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DAIRY SCIENCE

Vol. 43 October, 1960 Number 10

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THEME: MARKET MILK

DEPARTMENT OF DAIRY INDUSTRY

MONTANA STATE COLLEGE, BOZEMAN

NOVEMBER 15 to 17, 1960

JOURNAL OF DAIRY SCIENCE

OFFICIAL PUBLICATION OF THE
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PEOPLE AND EVENTS

E. S. Estel Dies

For 43 yr. E. S. ESTEL was secretary-manager of the National Dairy Cattle Congress at Waterloo, Iowa. He died August 3 at the age of 73.

Known personally by dairy cattle owners and showmen throughout the land, Estel was largely responsible for building the Dairy Cattle Congress into the world's leading dairy cattle show and farm machinery exhibition.

When he graduated from Iowa State University, in 1910, the Dairy Cattle Congress was a small, one-day celebration. After serving for a time on the Kansas State dairy staff, Estel returned to Iowa as assistant field secretary of the Iowa State Dairy Association. An untiring promoter, he assisted in establishing the Dairy Cattle Congress as a permanent show and was elected assistant secretary-manager, in 1915. Two years later he became secretary-manager.

From then until he retired, in 1958, the show prospered under his expert guidance and energizing force and, today, the eight-day exposition is the site of five national breed shows, the National Intercollegiate Dairy Cattle Judging Contest, the National 4-H Dairy Cattle Judging Contest, and the National FFA Dairy Cattle Judging Contest.

Many of the policies inaugurated at the Dairy Cattle Congress have become universally accepted in state and regional shows.

A dedicated servant to the entire dairy industry, Estel was one of the founders of the American Dairy Association and was chosen its first permanent secretary, serving from 1940 to 1947. For his contribution to the dairy industry, his portrait was hung in the Dairy Shrine Club at Waterloo, in 1953.

News from Connecticut

H. PATRICIA MACLEOD, Assistant Professor of Dairy Bacteriology, and R. G. JENSEN, Assistant Professor of Dairy Chemistry, have received a research grant from the Public Health Service to study the lipid metabolism of streptococci. The grant, which will continue for 3 yr., totals $16,000.

Nebraska News

The Department of Dairy Husbandry will sponsor a Buttermaker's Short Course, beginning October 10 and continuing through

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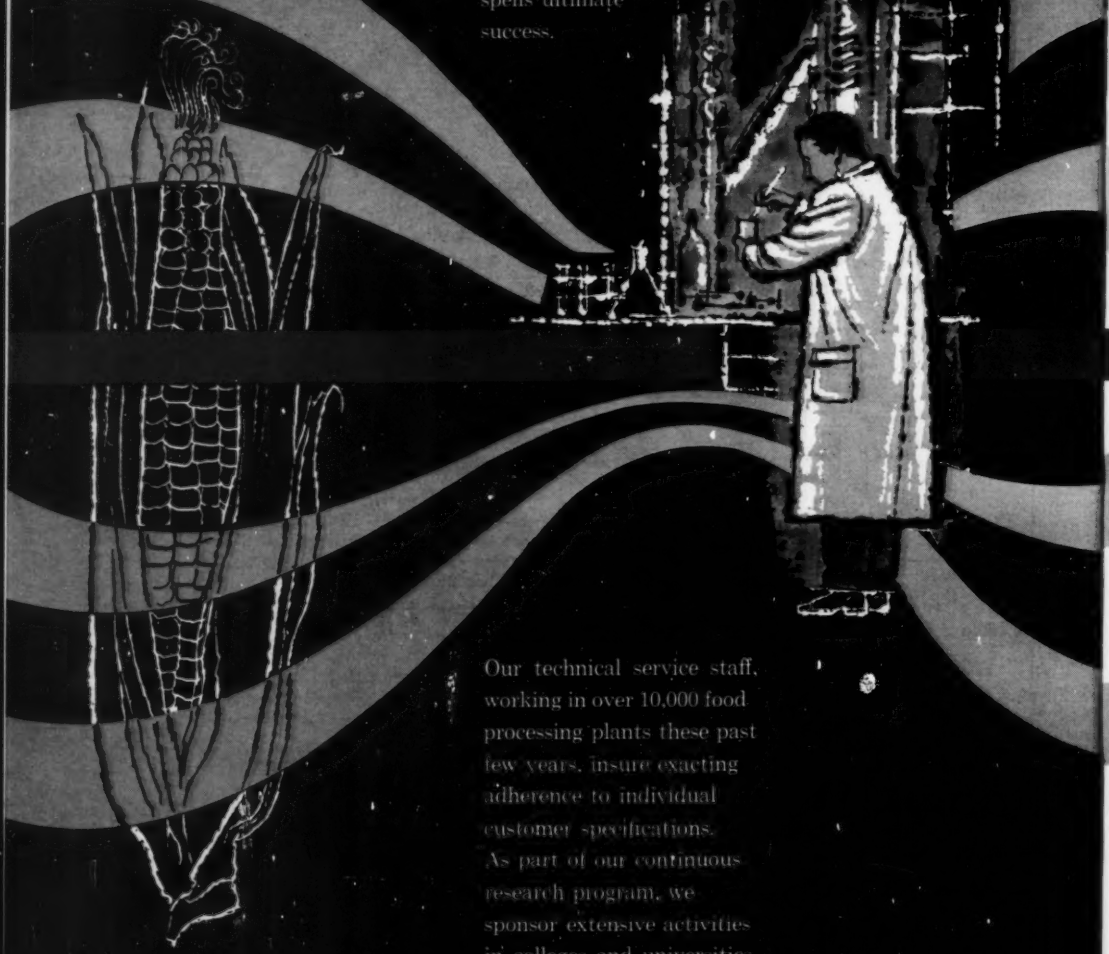

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October 14. It will be held in the Dairy Industry Building on the campus of the College of Agriculture at Lincoln. Aside from a planned program, the staff members of the Dairy Department will be available for conferences on individual problems.

Pennsylvania News

SUSUMU ADACHI, a Japanese dairy scientist, is conducting research at the Pennsylvania State University under a 1-yr. fellowship from the Rockefeller Foundation. Adachi is Associate Professor of Dairy Science at Tohoku University in Sendai, Japan.

He is working in the dairy manufacturing laboratory of the Department of Dairy Science in association with D.V. JOSEPHSON and STUART PATTON. He is also making a Japanese translation of the textbook Principles of Dairy Chemistry, by Jenness and Patton. The book, published in August, 1959, is the first textbook of its type published in the United States.

Adachi was the first researcher to show the conversion of lactose to lactulose in evaporated milk. Lactulose is a rare sugar. His special area of interest is browning deterioration of foods, especially sugar degradation.

Government Personnel Changes

W. I. PATTERSON, formerly chief, Dairy Products Laboratory, became on July 1 Assistant Director for Foreign Development, with headquarters in Washington, D. C.

W. I. Patterson

B. H. Webb

B. H. WEBB resigned from the National Dairy Products Corporation Research and Development Division to join the Eastern Utilization and Development Division as Chief of the Dairy Products Laboratory. He was formerly a member of the staff of this laboratory before joining the National Dairy Products Corporation. Dr. Webb has made significant contributions to the technology and physical chemistry of concentrated milk products. In 1943, he was awarded the Borden Gold Metal and $1,000 in recognition of his scientific contributions.

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Carl R. Brewer to New Position

CARL R. BREWER, formerly chief of the Research Division, U. S. Army Chemical Corps Research and Development Command, Washington, D. C., has been appointed chief of the Research Grants Branch, Division of General Medical Sciences, National Institutes of Health.

In his new position, Dr. Brewer will supervise the administration of the DGMS program of grants for research in the sciences basic to medicine and biology, in environmental and public health, and in certain clinical sciences. The Research Grants Branch also supervises the DGMS Russian Scientific Translation Program and the Clinical Research Facilities Program.

C. R. Brewer

Dr. Brewer was born in Indianola, Iowa, in 1912. He received the B.A. degree from Simpson College in 1934, and the Ph.D. degree in physiological bacteriology from Iowa State College in 1939. From 1939 to 1942, Dr. Brewer was a lecturer in bacteriology at McGill University, Montreal, Canada. In 1942, he went to the University of Maine as assistant professor of bacteriology. The next year he became a research chemist at Lederle Laboratories.

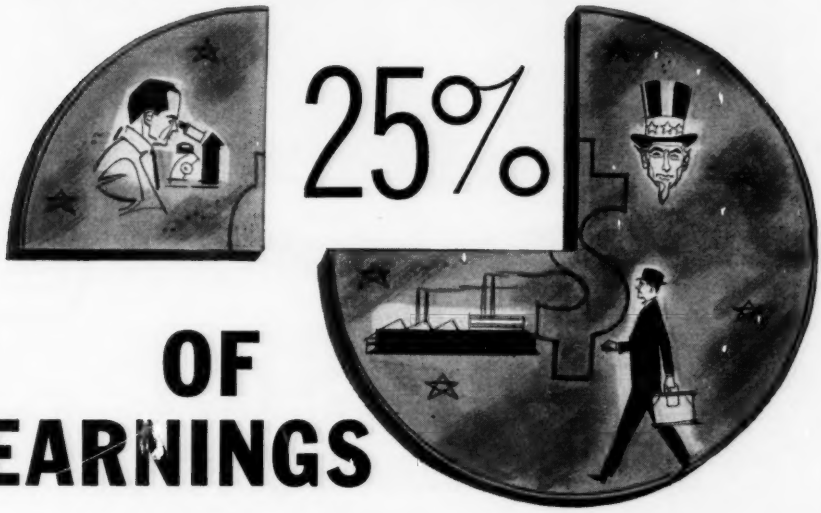
In 1944, Dr. Brewer was named chief of the Bacterial Nutrition Branch of the U. S. Army Chemical Corps Biological Laboratories, Fort Detrick, Frederick, Maryland. From 1946 to 1952, he served also as a part-time member of the graduate faculty of George Washington University. In 1953, he was named chief of the Allied Sciences Division at Fort Detrick, and in 1956 was appointed as chief of the Research Division, U. S. Army Chemical Corps Research and Development Command, Washington, D. C.

In 1945, Dr. Brewer received the War Department's Exceptional Civilian Service Award for developing and leading a program on the nutrition and physiology of pathogenic microorganisms. This program succeeded in developing some of the first chemically defined systems for the growth and metabolic study of highly infectious microorganisms, leading to improved specific knowledge of infectious disease.

Dr. Brewer is a member of the Sigma Xi and Epsilon Sigma fraternities, and a member of the American Society of Biological Chemists, the Society of American Bacteriologists, and the American Academy of Microbiology. His research interests include bacterial me-

FOR SANITATION RESEARCH

DIVERSEY INVESTS



OF EARNINGS

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tabolism and nutritional requirements and intermediary metabolism of pathogenic microorganisms, on which he has written a number of papers.

Cherry-Burrell Appoints J. H. Brinker Vice-President

J. H. BRINKER has been appointed executive vice-president of the Cherry-Burrell corporation. He held this same position with the J. I. Case Company, resigning last July.

J. H. Brinker

Prior to his affiliation with J. I. Case, Brinker served as general manager of A. O. Smith Corporation's Permaglas Division in Kankakee, and then as vice-president of A. O. Smith Corporation, Milwaukee. Before World War II, he was sales engineer at the Pfaudler Company, Rochester, New York.

A graduate of Evanston Township High School, Evanston, Illinois, 1932, Brinker received his A.B. in economics at the University of Rochester, New York, 1936. In 1947 he graduated from the Harvard University School of Business Administration with an M.B.A. degree.

During World War II, he served in the U. S. Navy from April, 1942, to September, 1946, and was separated from active duty as a Lieutenant Commander.

Borden's News

The retirement of M. A. GLASGOW, administrative vice-president of the Borden Foods Company, after almost 29 yr. of service, has been announced by T. O. HOFMAN, president.

Mr. Glasgow, who recently moved to Newton, New Jersey, joined Borden's in 1931 as a special automotive cost accountant in the

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company's general controller's office. In 1937, he was transferred to Borden's Dairy Delivery Company in California, where he worked on special audit reports. He returned to New York City in 1939 as sales statistician and subsequently was named assistant to the president of a division then known as the Borden Cheese Company. Mr. Glasgow was appointed executive assistant of the cheese operation in 1945.

When Borden's cheese and food products divisions were consolidated early in 1958, Mr. Glasgow was named divisional controller. In December, 1958, he was promoted to administrative vice-president.

R. C. WARREN has been named director of sales for the Central Division of the Borden Company, with headquarters in Chicago. He formerly was general manager of the company's Iowa Milk and Ice Cream Division, with offices in Rock Island, Illinois. Warren started with the Sturtevant Dairy Products Company in 1934, and when Borden's acquired Sturtevant he continued with the operation.

The Central Division, of which Warren becomes sales director, has responsibility for the manufacture and sale of Borden's milk and ice cream in Illinois, Iowa, Wisconsin, and parts of Indiana and Michigan. In addition, it supervises sales of cheese and other Borden food products in these states and several others.

R. C. BEYER has been named sales manager of the Central Illinois Region of the Borden Company's Central Division. His headquarters will be in Pekin, Illinois. He formerly was assistant manager of Borden's operation in the Des Moines, Iowa, area.

At the same time, G. A. WEBER was promoted from plant superintendent to production manager. C. G. KIRCHHOFF, formerly plant superintendent for Borden's at Barrington, Illinois, succeeds Weber as superintendent at Pekin.

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From the Secretary's Desk

The Secretary,
American Dairy Science Association,
32 Ridgeway Circle,
White Plains, New York, U.S.A.

Recently, I had the pleasure of visiting the United States and studying there the market milk, ice cream, and milk powder side of the Industry. Wherever I went, I was astounded at the kindness and hospitality shown by people everywhere in your country. I visited a number of dairy plants and was struck with the frank and open manner in which the techniques and problems associated with manufacture were discussed. Industry organizations, universities, and equipment manufacturers all assisted in a most helpful fashion. There is a great deal that we in Australia can learn from the United States Dairy Industry, not the least being the cooperative effort by all concerned to further the development and use of dairy products.

Towards the end of my visit, I attended the Annual Meeting of your Association at Logan, Utah, and I must compliment both the American Dairy Science Association and the University of Utah on the efficient organization of this conference. On the social side, the very pleasant barbecue and the magnificent performance of the Tabernacle Choir will long be remembered. The campus of the University was indeed a perfect setting, with the mountains around and, of course, the perfect weather.

Having been a reader of the JOURNAL for many years, I did enjoy meeting many people whom I knew well by name. Being involved in so many fields, my only regret was my inability to hear all the papers in which I was interested. Your thoughtfulness in presenting those from overseas with an International Guest ribbon was much appreciated by us all.

As a result of this visit, I have made many new friends and learned a great deal, thanks

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It is easy to create a great commotion with the water . . . easy to wash the pipe line itself, but this won't hold your count down if you let the receiver pail and trap with the connecting line accumulate contamination. Unwashed spots can quickly become dirty spots . . . they become zones of contamination. That's why Surge is so very fussy about washing them.

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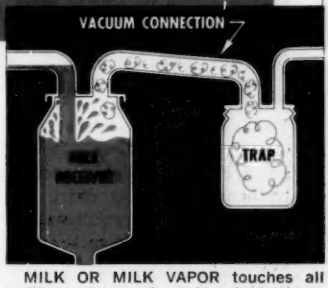
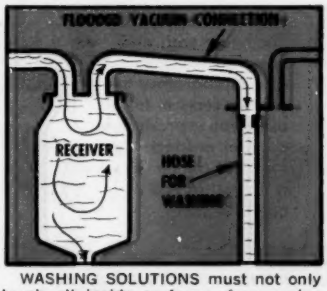


MILK OR MILK VAPOR touches all inside surfaces of a pipe line system during milking. Top of milk lines, bottom of receiver and dispenser covers . . . even vacuum connections to traps collect some milk film through splashing or condensation.



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to the people in the Dairy Industry of the United States. I can assure a warm welcome to any members of the Industry from your country who may visit Australia.

Yours sincerely,

DEREK SHEW
Paul's Ice Cream & Milk Ltd.
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Letter to the Editor

I have read with interest the opinions of several members of the A.D.S.A. regarding the publication of the Interpretive Summaries.

Is it not considered desirable that research workers bring the results of their work to the attention of as many people as possible who can utilize them? If so, does working in a union of scientists mean that whatever we do, say, or write must be intelligible to only another scientist? I believe we can better serve our association, industry, and nation if more individuals know us and our work. I think that these interpretive summaries are utilized by scientists to determine whether the article is of sufficient interest to warrant reading. On the other hand, the layman, by reading these summaries, may be encouraged to explore the scientific presentation.

Questions have been raised relative to the time required of scientists to write the summaries, and the financial aspect of publishing them. These elements, when considered in view of the time and money spent on doing the research work, would be infinitesimal and would be more than justified in view of the service they may render.

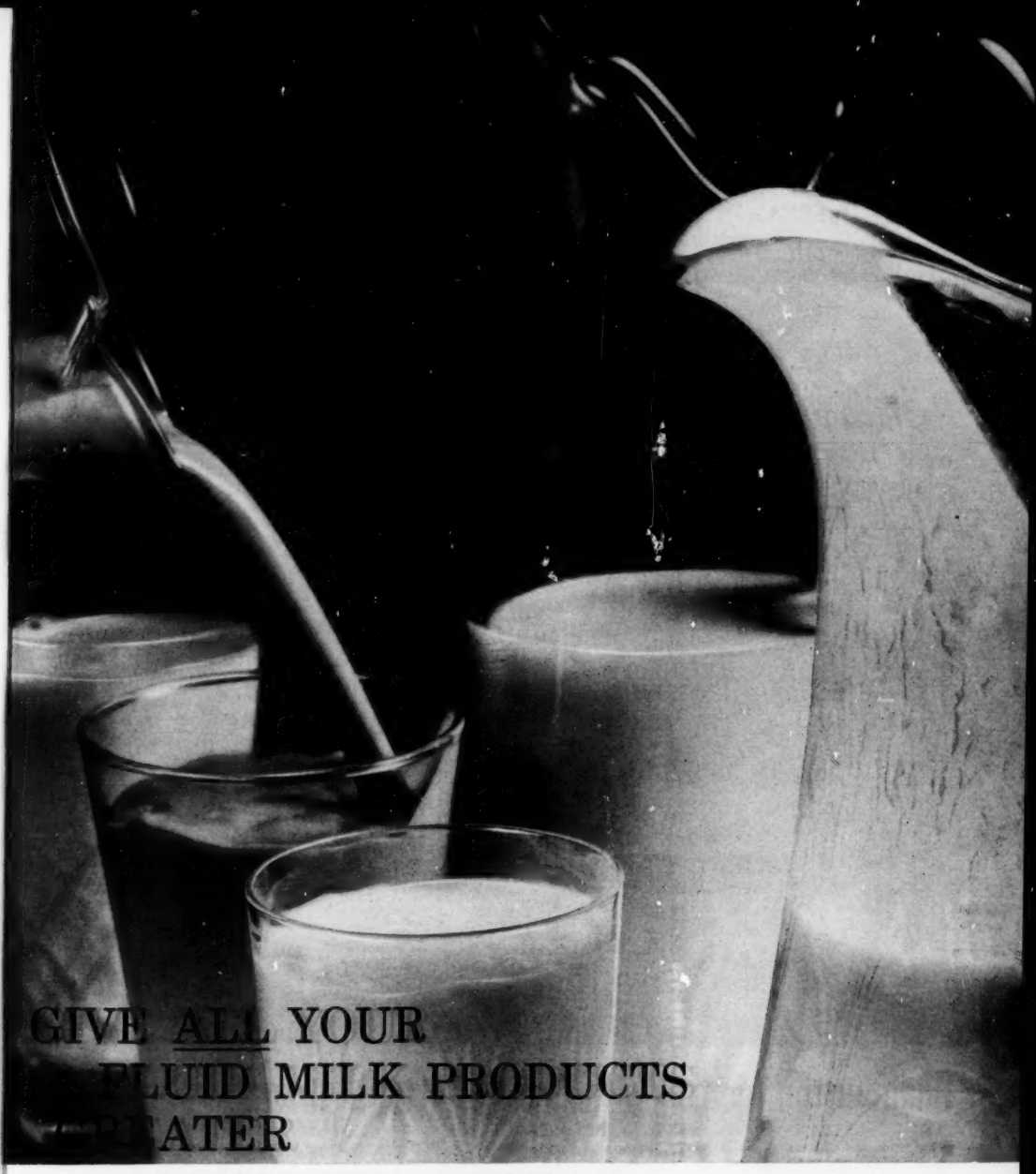
I support the continuation of the summaries as consistent with the objectives set forth by the committee on publication policy.

KHEM M. SHAHANI
Associate Professor
Department of Dairy Husbandry
The University of Nebraska, Lincoln

Dairy Technology Societies

Central Michigan—The fall program opened on the evening of September 21, at the High-Life Inn, Saginaw. Guest speaker was WORTH WEED, Foote & Jenks, Jackson. Mr. Weed's topic was Dairy Diplomatic Corps, and related to some of the problems encountered by the Dairy Society International.

Kansas—The September 12th meeting featured W. H. E. REID, Dairy Department, University of Missouri, as guest speaker. His topic was Views of Milk and Milk Products Factories in Several European Countries. His lecture was illustrated with a fine collection of slides, and was thoroughly enjoyable and highly informative.



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Philadelphia—New meeting place is the Drexel Institute—Picture Gallery Room, 31st & Chestnut Streets. New officers for 1960-61 are: G. D. WEBER, SR., president; E. W. COOK, vice-president; DUANE PULTERER, secretary-treasurer; A. F. ZIMMERMAN, assistant secretary; and THOMAS WADDELL, assistant treasurer.

Tri-Cities—Speaker at the September 13 meeting was D. R. FRAZEUR, Dairy Department, Purdue University, whose topic was That's Using the Old Nose, dealing with off-flavors in dairy products.

Tri-State—First meeting of the season was held September 21 at the Fort Steuben Hotel Ballroom, Steubenville, Ohio. Speaker was EDWARD GRAVER, executive secretary, Ohio Dairy Products Association. Officers announced for the coming fiscal year are: HERMAN BOLAND, president; ED MARXEN, first vice-president; JIM WILLIAMS, second vice-president; DRUSILLA HOKE, secretary-treasurer; and C. J. HONER, corresponding secretary.

Western Michigan—Program speaker for the September meeting was HARRY BOWSER, Sales Development Manager, Dairypak, Incorporated, speaking on Meeting the Sales Challenge of the Sixties. The meeting was held at Finger's Cafe, Grand Rapids, September 19.

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TRAINING FOR LEADERSHIP

Prepared by

THE PERSONNEL TRAINING MANUAL PREPARATION COMMITTEE
THE AMERICAN DAIRY SCIENCE ASSOCIATION

H. B. HENDERSON, University of Georgia, Athens, Georgia, Chairman
F. C. EWBANK, Michigan Milk Producers Association, Imlay City, Michigan
I. A. GOULD, The Ohio State University, Columbus, Ohio
FARNUM GRAY, Southern Dairies, Charlotte, North Carolina
H. F. JUDKINS, American Dairy Science Association, White Plains, N. Y.
H. F. WILLIAMS, Carnation Company, Los Angeles, California

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Section I Objectives and Requirements of the Program

Section II Training Program Schedules for Plant Operation

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2. Plant operations
3. Laboratory
4. Engineering and maintenance
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Section III Management and Development

1. Developing skills in personnel management
2. Developing skills in quality control and cost

Many study projects are outlined which are not only essential to management development but may result in savings to the plant that will more than pay the cost of training.

Appendix

1. List of professional and trade organizations
2. List of reference books, booklets, and periodicals

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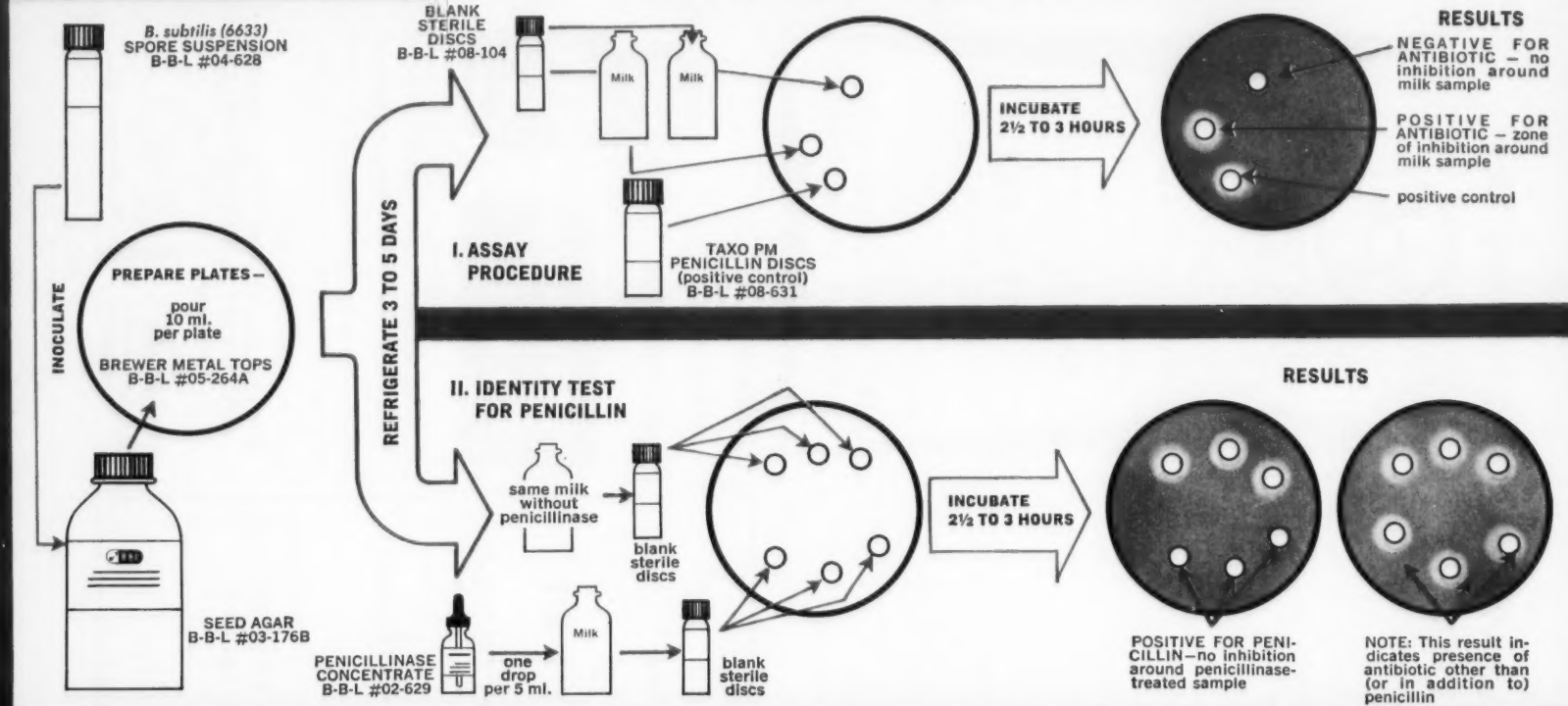
DETECTION OF PENICILLIN IN MILK



The presence of antibiotics in milk following mastitis therapy in cows has created serious public health problems and caused technical difficulties within the dairy industry. A rapid, practical laboratory procedure to assist regulatory agencies and the dairy industry in solving these problems was described by Arret and Kirshbaum.* This procedure employs rapid growth of a sensitive strain of *B. subtilis* for assaying the presence of antibiotics in milk and for determining its identity with penicillin. Inhibition of growth by the presence of as little as 0.05 unit of penicillin per ml. of milk sample is detectable within 2½ hours.

In answer to many requests for information about the availability of B-B-L products for this simplified procedure, the B-B-L Development Laboratory has prepared this TECHNICHART. It graphically illustrates the basic procedure, showing the materials necessary—**all of which are available from B-B-L.** A complete brochure with detailed technique and product listing is available upon request. No. 13

*Arret, B., and Kirshbaum, A.: J. Milk and Food Technol. 22:329, 1959.

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LABORATORY STUDIES ON HIGH TEMPERATURE–SHORT TIME STERILIZED EVAPORATED MILK. I. EASILY CONSTRUCTED ECCENTRIC FALLING BALL TYPE BOMB MICROVISCOMETERS SUITABLE FOR CONTAINING BIOLOGICAL MATERIALS DURING STERILIZATION AND STORAGE

ABRAHAM LEVITON AND M. J. PALLANSCH

Dairy Products Laboratory, Eastern Utilization Research and Development Division, USDA, Washington 25, D. C.

SUMMARY

A simple eccentric falling ball type bomb microviscometer is described. This instrument, which lends itself to easy construction in large numbers, is suitable for containing, sterilizing, and storing of milk, milk concentrates, and other biological liquids. Samples as small as 0.01 ml. may be processed and stored. Viscosity measurements may be made before and after sterilization and during storage. The operational characteristics of these microviscometers have been considered in the light of the empirical and semiempirical relationships which apply to falling ball viscometers of the eccentric type. These relationships have been shown to extend to the very small diameter tubes and spheres employed in the construction of the bomb microviscometers. The effect of the angle of inclination on the instrument constant has been determined and this effect furnishes a basis for the rejection of malfunctioning viscometers.

Food products and biological materials, in general, which are to be preserved for any length of time, require sterilization. When this is brought about by the employment of high temperatures, deleterious physicochemical changes in the material may occur. An important criterion of quality modification is change in the body or consistency of the product. A suitable related measurement is that of the rate of shear attending the application of a definite shearing stress.

The rate of shear may be proportional to the shearing stress. Fluids for which this relation holds are classified as Newtonian and truly viscous. All other fluids are non-Newtonian and are by definition only apparently viscous. For these, the viscosity index is not independent of the rate of shear but varies with it and/or with the history of the sample.

The need for adequate laboratory techniques in gelation studies on concentrated sterile milk led to the development of a microviscometric technique particularly suitable to such studies. Inasmuch as the microviscometric technique is one which may have a broad biological application in all fields where the determination of the viscosity of micro samples is desired, the construction and operation of the viscometer are discussed, along with certain relationships necessary for the intelligent use of the instrument.

APPARATUS AND METHODS

In high temperature–short time sterilization, the bringing-up time must be quite rapid in comparison with the short holding time. Since it was desired to sterilize samples in the measuring device, this criterion limited the material

Received for publication May 4, 1960.

employs rapid growth of a sensitive strain of *B. subtilis* for assaying the presence of antibiotics prepared this TECHNICHART. It graphically illustrates the basic procedure, showing the materials

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for construction of the microviscometers to thin-walled capillary tubing. The microviscometer was, therefore, made to consist of a thin-walled capillary tube and a small glass bead operating on the principles of the well known Höppler viscometer (2); this permitted measurements on samples as small as 0.01 ml. to be made. The microviscometer also served as a bomb in which samples could be sterilized at temperatures above their boiling point. This permittted viscosity measurements to be made on the samples before and after sterilization and during storage, with a minimum disturbance of the sample.

The microviscometer was constructed from tubing, the bores at the ends of which approximated 0.45 mm. Measurement of bore diameter was made by the use of a tapered beading needle and a microscope equipped with an ocular micrometer. For relatively precise work, tubes were selected approximately 5 cm. in length, the bores at the ends of which were the same to within 0.003 mm.

Glass beads were obtained from the Minnesota Mining and Manufacturing Company[1] in sizes ranging from 28-470 μ in diameter. Preference in this investigation was given to beads in the range 250-375 μ. Rolling of the beads during transfer was prevented by impregnating the surfaces on which the beads were lodged with glycerol. Bead diameters were determined microscopically and transfers made by means of a sharp-pointed tweezer. Distorted beads were largely eliminated by a procedure in which beads were permitted to roll on the smooth base of a rectangular optical cell inclined at an angle of approximately one degree. Only those beads were selected which traversed the cell from one edge to the opposite one in the shortest time.

Tubes were cleaned in a conventional manner by aspirating through them in succession hot detergent solution, water, and acetone. Capillary action was used to partially fill each tube with sample. A leakproof closure of the clean end was made by sealing around a glass bead positioned in the end of the tube. The trapped air cell in the tube was removed by centrifuging at approximately 500 $\times$ gravity. Through the remaining open end a bead of predetermined diameter was forced into the sample, using a pipette probe. The wall of the viscometer located above the liquid level was cleaned by inserting a drawn glass capillary tube to a point above the liquid level, immersing the tube in water, and aspirating. The clean open end was then sealed and expanded slightly to give an air-tight seal. The position of the fluid thread in the tube was reversed by centrifuging and, simultaneously, trapped air was eliminated.

To measure viscosity, the tube banded to a thermometer was oriented with its axis parallel to the thermometer thread. The thermometer inserted into a 500-ml. cylindrical water-bath was aligned in a vertical position by means of a stopper and a mark at the base of the cylinder. The water-bath was then strapped on to the slant side of truncated blocks of known side angle. At the time a measurement was made, the block in front of a titrator lamp was rotated 90° about a horizontal axis. The time required for the bead to move 2.25 cm., or multiples as fractions thereof from a point 0.7 cm. from the base

[1] It is not implied that the U. S. Department of Agriculture recommends the above company or its product to the possible exclusion of others in the same business.

of the tube, was noted. Densities needed for calculation purposes were determined pycnometrically, and the viscosity of glycerol solutions needed for standardization was measured with a Bingham viscometer (1).

The microviscometer was emptied of calibrating fluid without removing the bead, by first severing the end portions of the viscometer, constricting the open ends, and removing the glycerine by suction. The cleaned, dried capillary tube was then filled with sample in the manner already prescribed.

RESULTS AND DISCUSSION

Viscometer constant. The principles underlying the use of viscometers of the eccentric fall type are well known (5). The high precision instrument, the Höppler falling ball viscometer, is widely used. Governing its use is the empirical equation:

(1) $\eta = kt\ (\rho_b - \rho_f)$ also written

(2) $\eta = \frac{C}{v}\ (\rho_b - \rho_f)$

These equations also govern the use of the microviscometer. In the equations η is the viscosity, ρ_b and ρ_f are, respectively, the densities of the spherical bead and fluid, t is the transit time between two marks on the viscometer, 10 cm. apart in the Höppler instrument, v is the velocity of fall, and k or C are instrument constants. The determination of the instrument constant does not lend itself to treatment based on theoretical hydrodynamics. Dimensional analysis, however, provides a clear insight into the relationships involved (3, 5).

The viscometer constant may be shown to be proportional to the square of either the bead or tube diameter, and to a function of the ratio between the bead diameter and the tube diameter, thus:

(3) $C = f(\frac{d}{D})\ d^2 g\ (\rho_b - \rho_f) \cos \theta$

The symbols d and D represent, respectively, the diameters of the bead and tube, $f(\frac{d}{D})$ is a function of the dimensionless variable $\frac{d}{D}$, g is the gravitational constant, and θ is the inclination of the tube to the vertical. The function, $f(\frac{d}{D})$ is independent of either d or D and, therefore, if evaluated under one set of conditions it should be valid under any other set.

Figure 1 shows two graphs, one referring to the Höppler instrument (5), the other to the microviscometer. The log of the instrument constant k is plotted against the log of the difference between the tube and bead or ball diameter. A linear relation holds for small values of D-d. The slope of the line is within experimental error the same, both for the microviscometer and the Höppler instrument. The microviscometer constant k in the region of small D-d is equal to $0.006\ D^{-1/2}\ g\ (D\text{-}d)^{2.5}$. The corresponding equation for the Höppler instrument in good agreement contains the constant 0.0057 in place of 0.006. The curves pass through a maximum located at approximately the same value of the

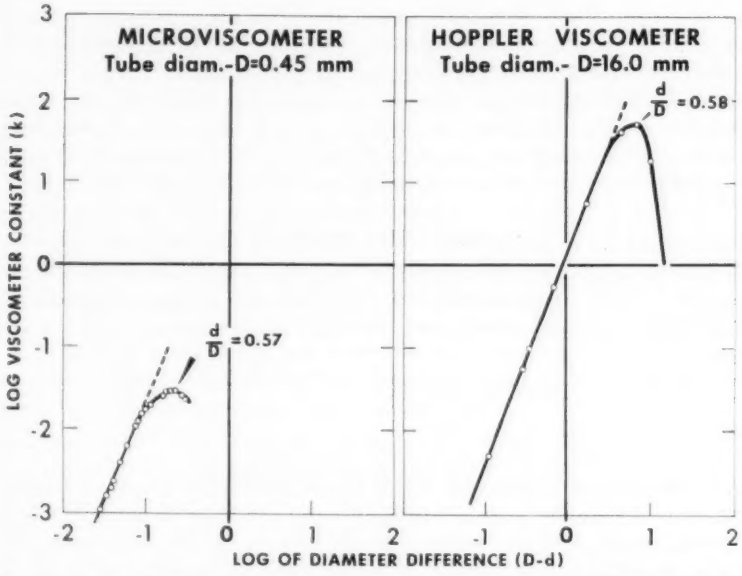

FIG. 1. The relationship between the instrument constant and the difference in diameter between the tube and ball.

ratio $\frac{d}{D}$. The ratio between the maximum values of k is equal to the square of the ratio between the tube diameters.

The relationships which have been noted are precisely those which would have been anticipated on the basis of dimensional analysis. Beyond the maximum in the region of small values of the bead diameter, k is proportional to the square of the bead diameter. This relationship is shown in Table 1, and begins to prevail when the bead diameter is about one-fourth the tube diameter. The rate of fall is then about one-sixth that which would have been observed had the fall been concentric and according to Stokes' Law.

The graphs are useful for estimating the time of transit, and they therefore serve as an aid in the selection of beads and tubes with respect to their diam-

TABLE 1

Relationship between viscometer constant and bead diameter for small diameters

Bead diameter (d) (*mm.*)	Tube const. (k) $\times 10^3$	$\frac{k}{d^2}$ — experimental	$\frac{k}{d^2}$ — calculated[a]
0.085	0.625	0.865	5.35
0.096	0.795	0.865	5.35
0.106	0.955	0.865	5.35
0.120	1.20	0.830	5.35
0.141	1.57	0.793	5.35

[a] Calculated according to Stokes' Law.

eters, in order to obtain transit times which fall within reasonable limits. For tubes 0.46 mm. in diameter, a minimum transit time is obtained of 3.7 sec. per centimeter per centipoise per unit density difference. This occurs when the bead diameter is about 0.26 cm. With smaller and larger beads, greater transit times are obtained. For constant $\frac{d}{D}$ the time will increase in proportion to the square of either d or D.

Type of motion executed by the bead. The force acting on the bead is proportional to the angle of inclination of the tube to the vertical. The product of the time and the cosine of the angle of inclination should, therefore, be constant. The results in Table 2 show, however, that the product increases as

TABLE 2

Variation of the time required for the bead to move 2.25 cm. with variations in the angle of inclination

Bead diameter (d)	Inclination (θ)	Time (t)	$t \cos \theta$
(mm.)	*(degrees)*	*(sec.)*	*(sec.)*
0.276	10.7	27.5	27.2
	29.6	34.0	29.5
	45.0	46.8	33.0
	60.4	79.5	39.2
	79.3	253.0	46.6

Tube diameter 0.490 mm.; temperature 30° C.; viscosity of glycerol solution, 5.47 centipoises.

the angle is increased. Observation under a microscope showed that the type of motion executed by the bead changed from a glide at an angle of approximately 10° to a progressively increasing roll as the angle was increased. The transition from a roll to a glide occurred at an angle which for any one tube was independent of the bead diameter and the viscosity of the fluid. The change in the type of motion would account qualitatively under ideal conditions for the observed relationship between velocity and the angle of inclination, as Weber (3) has shown in connection with the Höppler instrument.

A basis for rejection of viscometers. In Figure 2 the ratios between bead velocities at the two angles 29.6 and 10.7° are plotted as abscissae, and the frequency of their occurrence as ordinates. A skewed distribution is obtained. The most probable value of the ratio lies in the range 0.795-0.825. The arithmetic mean is 0.800 and the standard deviation is $\pm$ 0.03. A hypothetical curve representing a normal frequency distribution has been superimposed on the bar drawing to emphasize the skewness. Abnormally low ratios between the velocities at the two angles are caused by bead imperfections and/or microscopic debris, and abnormally high values by distorted beads. Microviscometers functioning abnormally are largely eliminated and accuracy is enhanced if selection is based on the requirement that the ratio of velocities at the two stated angles should fall within the range of two standard deviations. The reproducibility of values obtained under these conditions is quite good (see Table 3). The estimated percentage deviation for a large number of measure-

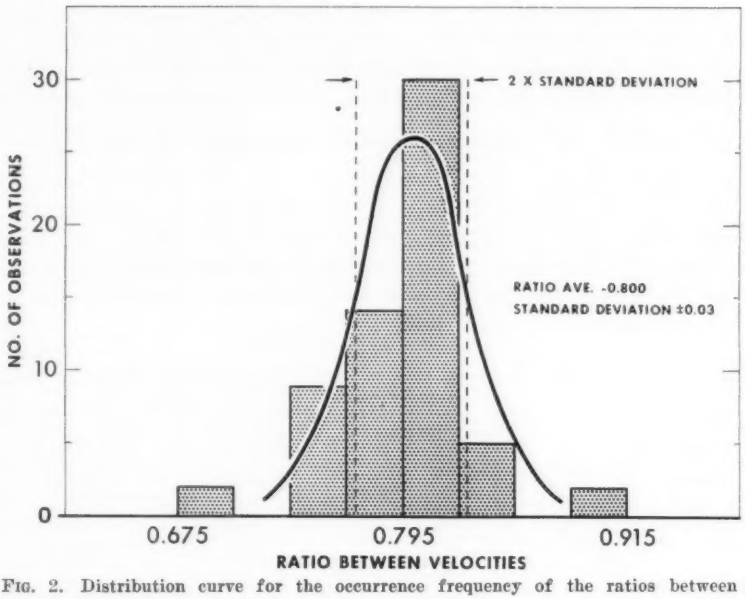

FIG. 2. Distribution curve for the occurrence frequency of the ratios between bead velocities at the two angles, 29.6 and 10.7°.

ments made on water and on glycerol solutions varying in viscosity from 0.8 centipoise to 35 centipoises was 0.3%. This estimate is based on the assumption that the percentage deviation is independent of the viscosity. The measured and literature viscosity values (5) agree quite closely. In one series of measurements for which the data are not shown, the ratio between the viscosities of a glycerol solution at 25 and 30° C. as determined with 15 different viscometers averaged 1.20 ± 0.01 centipoises, compared with the literature value (4) of 1.198.

The microviscometer has been developed chiefly for investigational work on evaporated milk. It should, however, find general application. Whenever extremely small volumes of material are available for measurements or proc-

TABLE 3

Comparison of viscosity values at 30° C. for glycerol solutions

Glycerol concentration	Viscosity microviscometer[a]	Literature values[b]	Difference
(%)	*(centipoises)*	*(centipoises)*	*(%)*
46.12	3.48	3.568	−2.5
54.47[c]	5.35	5.352	0.0
64.89	9.99	9.956	+0.3
75.00	20.8	21.68	−4.0
80.00	33.5	34.92	−4.0

[a] Bead diameter 0.270 mm., tube diameter = 0.416 mm., θ = 10.7°.

[b] M. L. Sheely. Ind. and Eng. Chem., 24: 1060. 1932.

[c] Solution used for standardization.

essing, whenever flash sterilization or pasteurization is required, and whenever changes in viscosity on storage are to be followed with minimum disturbance of the sample, the microviscometer has much to recommend it, provided the ultimate in precision is not required in viscosity measurements.

REFERENCES

(1) BINGHAM, E. C. Fluidity and Plasticity. p. 285. McGraw-Hill Book Co., Inc., New York. 1922.
(2) HÖPPLER, F. The Eccentric Fall of Spheres Through Liquids or Gases in Cylinders. Z. tech. Phys., 14: 165. 1933.
(3) HUBBARD, R. M., AND BROWN, G. G. The Rolling Ball Viscometer. Ind. Eng. Chem., Anal. Ed., 15: 212. 1943.
(4) SHEELY, M. L. Glycerol Viscosity Tables. Ind. Eng. Chem., 24: 1060. 1932.
(5) WEBER, W. Systematische Untersuchung des Fallkugelviskosimeters mit geneigtem Fallrohr. Kolloid Z., 147: 14. 1956.

FORMATION OF A FAT PROTEIN COMPLEX IN MILK BY HOMOGENIZATION

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SUMMARY

A fat-protein complex which sediments in a centrifugal field is produced in milk by homogenization. Fat-protein complex formation is found to increase with increasing homogenizing pressure; with increasing fat content; with an increase in concentration above 31% total solids (T.S.); and with increasing calcium concentration up to a limit of 10 m*M* added calcium. In a suspending medium of increased density produced by adding sucrose, the fat-protein complex formed by homogenizing concentrated milk of more than 31% T.S. is found to consist of a single entity. In nonconcentrated milk, the fat-protein complexes have a range of fat-nitrogen ratios.

Experiments to determine the manner in which the fat-protein complex is formed indicate that conditions existing at the homogenizer valve at the instant of homogenizing are responsible for complex formation. Ultracentrifuge analyses of the protein moiety of the fat-protein complex show it to be casein.

Currently, in the Dairy Products Laboratory of the Eastern Utilization Research and Development Division, the unique physical properties of foam-dried whole milk powder (7) are being investigated. Upon centrifuging a reconstituted sample of this powder, fat was found associated with the casein in the sediment. Investigation showed that a fat-protein complex was formed during the homogenization step preceding drying. Since the amount of protein-fat complex would conceivably account for some of the physical properties of foam-dried powder, fat-protein complex formation was subjected to further research.

The proteins normally adsorbed on the fat globules have been the subject of much study (6). Brunner, Duncan, and Trout (2) found that the proteins bound on the fat globules of homogenized milk were different from the proteins in nonhomogenized milk. In these earlier studies attention was given to the protein-fat complex which floated in milk subjected to centrifugation. This paper, in contrast, is concerned only with those fat-protein interaction products of sufficient density to sediment in milk subjected to high centrifugal fields.

This paper describes formation of sedimentable fat-protein complexes in terms of: (1) homogenization pressure, (2) total solids concentration, and (3) fat to solids-not-fat ratios. Experiments designed to discern the mechanism responsible for the fat-protein interaction also are described.

EXPERIMENTAL PROCEDURE

Material. All milk used in this study was obtained from mixed herds located at the Beltsville Experiment Station of the U. S. Department of Agriculture. The raw milk was routinely standardized and pasteurized at 145° F. for

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30 min. Concentrates were prepared from the pasteurized milk by using a single-effect Rogers Pan.[1]

Homogenization. Milk and milk concentrates were homogenized at 145° F. using a single stage of a Manton Gaulin Model 75 homogenizer [1] equipped with a Dyna Jet Homogenizing valve.[1]

Reconstitution. After homogenizing, the concentrates were diluted with distilled water to the solids content of the unconcentrated milk. The reconstituted and nonconcentrated milks were both held at 36° F. for 48 hr. to allow the internal milk systems to come to equilibrium. This holding period was necessary to secure reproducibility of quantitative measurements. The samples were warmed to 104° F. to put the fat in the liquid state, and centrifuged at this temperature.

The samples were centrifuged in a Spinco Model L[1] preparatory centrifuge using a No. 40 rotor. They were centrifuged at 150,000 × G (av.) for 90 min. Nitrogen depletion-rate measurements on nonhomogenized milk indicated that this centrifugal force was almost six times greater than necessary to sediment the caseinate complex.

Nitrogen determinations were made by the micro-Kjeldahl method of the A.O.A.C. (1). Fat analyses were made by the Mojonnier method.

For examining the protein of the fat-protein-fat complex, a Spinco Model E[1] ultracentrifuge and an Aminco[1] electrophoresis apparatus were used. The protein was suspended in a veronal buffer, pH 8.4, total ionic strength 0.1, containing 0.05 *M* NaCl.

Analytical technique. After centrifuging, a pellicle of fat was found at the top of the centrifuge tube, the amount depending upon the homogenizing pressure. This fat was discarded. The supernatant liquid was collected and analyzed for nitrogen. The sediment was recovered and analyzed for fat and nitrogen. The sediment was always in the form of a firm pellet in the bottom of the centrifuge tube. These pellets were ground in a mortar and weighed samples taken for fat and moisture determinations. It was found that the wet sediment could be easily dissolved for fat analysis by adding 10 ml. of ethylenediaminetetracetic acid (E.D.T.A.) solution (eq. to 0.6 mg. Ca^{++} ml.) to the Mojonnier[1] flasks containing the weighed sample (1-2 g.). The moisture content was found by drying the wet sediment in a vacuum oven for 12 hr. at 105° C. An aliquot of the dried sediment was taken for nitrogen analysis. Fat analysis by the Mojonnier method could not be made on the dried sediment.

RESULTS

Extent of complex formation. The effect of homogenization pressure on formation of a sedimentable fat-protein complex is shown in Figure 1. The milk contained 12.4% T.S., of which 3.3% was fat. The different concentrations are indicated as 1:1 (unconcentrated), 2.5:1 (31% T.S.), 3:1 (37.2% T.S.), and 4:1 (49.6% T.S.). At all concentrations the ratio of T.S. to fat is the

[1] It is not implied that the U. S. Department of Agriculture recommends the above company or its product to the possible exclusion of others in the same business.

same. The graphs represent the average of three determinations. The values for a 2:1 concentration are shown in Figure 2. Likewise in Figure 1, data for homogenizing pressures of 1,000 and 6,000 p.s.i. are shown but are not included in Figure 2.

Figure 1 shows that increasing the homogenizing pressure causes an increase in the formation of a sedimentable fat-protein complex at all concentrations. It is also seen that the homogenizing pressure range normally used in industry produces the fat-protein complex.

In Figure 2, the fraction of the fat sedimenting is plotted versus the T.S. concentration for three of the homogenizing pressures studied. It is observed that above a concentration of 31% T.S. at the fat level used, a significant increase in the amount of fat bound to the protein occurs. Because of the deviations in the data at 37% T.S. this concentration is considered to be at or near the critical point for the occurrence of some factor responsible for the increased binding of the fat to the protein. More information regarding this factor is presented later.

Identity of the protein in the complex. The identity of the protein involved in the complex is established in part or wholly by several methods.

a. Amount of protein required to sediment the fat. By reference to Figures 1 or 2 it can be seen that when a 4:1 (49.6% T.S.) concentrated milk is homogenized at 8,000 p.s.i. about 75% of the fat sediments. Since the milk

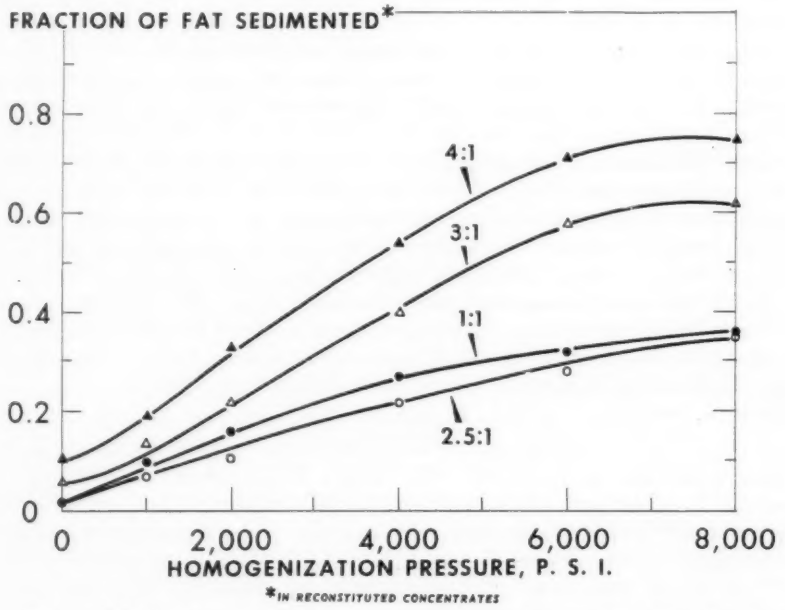

FIG. 1. Effect of homogenization pressure on fat-protein complex formation in milk and concentrated milks.

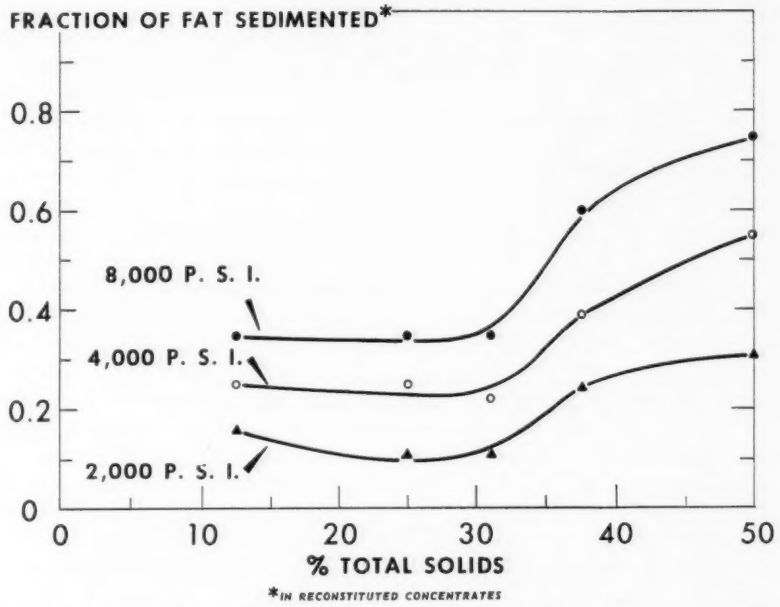

FIG. 2. Fat-protein complex formation as a function of concentration at different homogenization pressures.

contains 3.3% fat, about 2.5 g. (75%) of fat is sedimented from a 100-g. sample. The density of the supernatant was 1.026 g/ml. This would be a limiting minimum value for the density of the complex, i.e., with this density it would remain suspended rather than sediment. Assuming the density of milk fat to be 0.93 g/ml, and using a value of 0.72 as the apparent specific volume of the protein, 0.78 g. is the calculated amount of protein required to produce a complex of density 1.026 g/ml. If the milk contained 3.5 g. protein of which 80% was casein, the 0.78 g. of protein is slightly greater than the amount of whey proteins present. Since the complex readily sediments instead of remaining suspended, and because higher fat to protein ratios have been observed (see Figure 4), casein must be a part of the complex.

b. The fat is removed by resuspending the complex in a solution of E.D.T.A., but is not removed in warm water. Also, the calcium-nitrogen ratio of the fat-protein complex is the same as that of the calcium caseinate complex (4). Both of these facts imply that the calcium caseinate complex is involved in the fat-protein interaction.

c. Ultracentrifuge data. To establish the identity of the protein in the sedimenting fat-protein complex, the complex was sedimented from a reconstituted 4:1 concentrated milk which had been homogenized at 8,000 p.s.i. It was washed two times by dispersing it in distilled water with a Waring Blendor. The washed complex was then dispersed in distilled water by blending, and

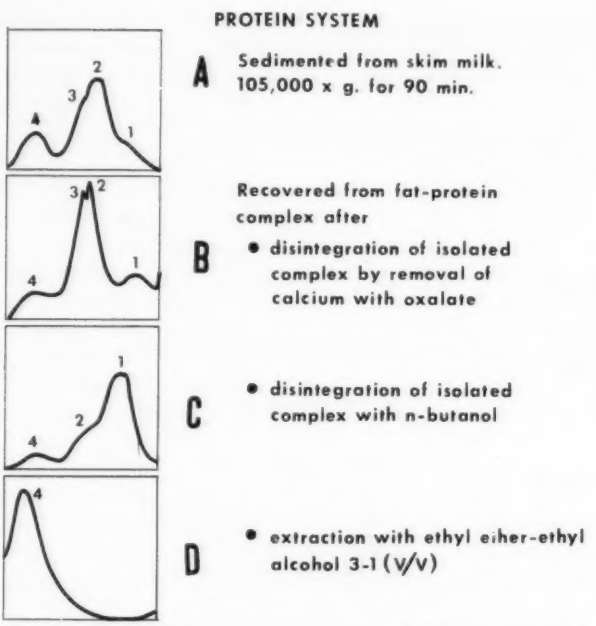

FIG. 3. Ultracentrifuge patterns of protein involved in complex 1, 2.

sufficient sucrose added to raise the specific gravity to 1.09. The solution was centrifuged and the fat-protein complex recovered by flotation. The flotated complex was dispersed in distilled water and washed two times with distilled water. The isolated complex had a fat/nitrogen (F/N) ratio of 9.1 : 1 and a calcium/nitrogen (Ca/N) ratio of 0.216. The protein entity was separated from the complex by several methods noted below, and its ultracentrifugal and electrophoretic properties compared with those of casein that had not been complexed.

The uncomplexed casein was obtained by centrifuging unheated skimmilk at 105,000 × g for 90 min. The ultracentrifuge patterns of the respective protein systems dissolved in veronal buffer, pH 8.4, 0.1 ionic strength, are shown in Figure 3. The photographs were all taken at 102 to 104 min. after sedimentation began. The temperature of centrifugation was from 20 to 27° C.

Figure 3-A is the ultracentrifuge pattern of the casein obtained from skimmilk, as described above.

Figure 3-B is the protein system obtained by treating the complex with E.D.T.A. or oxalate ion as ammonium oxalate, followed by centrifugation to remove the fat and calcium oxalate. The solution was then dialyzed against several changes of buffer to remove excess oxalate ion. Except for better resolution of sedimenting components in B, the patterns of B and A are essentially the same.

Figure 3-C is the ultracentrifuge pattern of the protein system of the complex recovered after disintegration in *n*-butanol. This treatment appears to have shifted the bulk of the faster-sedimenting components to that of the component marked 1.

Figure 3-D is the ultracentrifuge pattern of the protein system recovered from the complex by extracting it three times with ethyl ether and ethanol (3:1-v/v). This treatment destabilizes most of the faster-sedimenting material. The latter sedimented rapidly at the beginning of centrifugation and formed a clear gel in the bottom of the centrifuge cell. It could not be redispersed as a stable solution in veronal buffer.

Although the protein system in the complex is predominantly casein, the manner in which it is extracted from the complex alters either the number of sedimenting boundaries or the distribution of material in the sedimenting boundaries.

Mode of interaction. To determine how the fat is complexed with the calcium caseinate, the following experiments were performed:

a. Butter oil was homogenized into milk dialysate at 8,000 p.s.i. The dialysate was prepared by dialyzing distilled water against a large volume of milk. This homogenate was then heated to 145° F. and mixed at a concentration of 3.3% with skimmilk (also heated to 145° F.) and with skimmilk which had been homogenized at 8,000 p.s.i. In neither experiment was a sedimentable

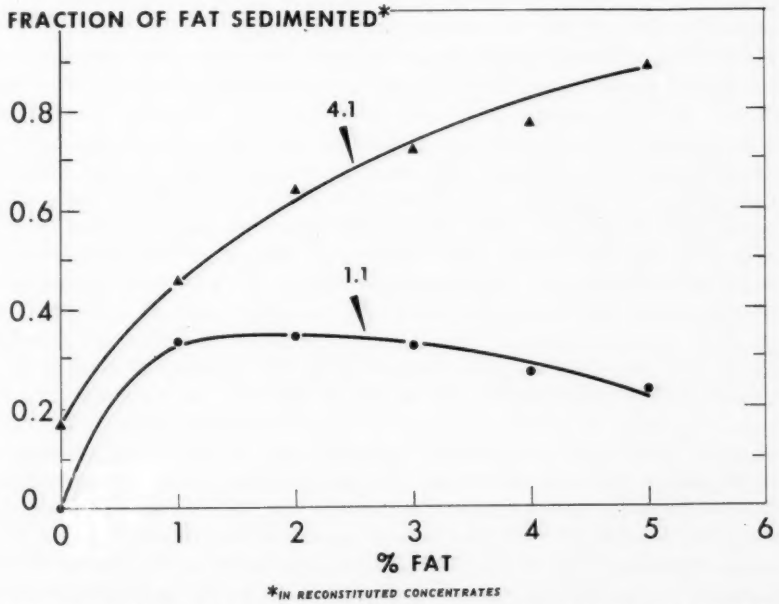

FIG. 4. Fraction of fat sedimented in milks and concentrated milks containing different fat contents. Homogenization pressure, 8,000 p.s.i.

fat-protein complex formed. This experiment shows that neither fresh fat surfaces nor the fineness of dispersion are sufficient to cause the fat to enter the protein phase.

b. Butter oil was homogenized directly into skimmilk at a concentration of 3.3%. The fat-protein complex was formed. In conjunction with the previous experiments, this shows that the interaction depends upon the conditions which exist at the homogenizer valve at the instant of homogenizing. It further illustrates that the fat globule membrane is not necessary for the formation of the complex.

c. Mineral oil was homogenized into skimmilk at a concentration of 3.3%. The fat-protein complex was formed. Since mineral oil does not contain any reactive or polar groups, the binding force must be by van der Waals forces between hydrocarbon sites on the caseinate complex and the fat. Because such binding occurs only during homogenizing, homogenizing must distort the caseinate micelle and expose hydrocarbon sites not otherwise available. The extent of such distortion and, therefore, the amount of fat bound would depend upon the homogenizing pressure.

Complex formation as a function of fat content. The previous experiments were all conducted on milk containing 12.4% T.S. and 3.3% fat—or some concentration of this milk. The effect of different fat content on complex formation was determined by the following experiment. A single lot of milk was separated and the cream diluted to 10% fat with the skimmilk. One part of the skimmilk was divided into six samples and sufficient 10% milk added to each sample to give a range of fat content of from 0 to 5%. The second lot of the skimmilk and the remaining 10% milk were concentrated to 32% solids-not-fat.

The concentrated skimmilk was divided into six lots and sufficient concentrated 10% milk (now concentrated to 39% fat) was added to the different samples to give a range of fat content of 0 to 20% fat. The samples were heated to 145° F. and homogenized at 8,000 p.s.i. The results are shown in Figure 4. In nonconcentrated milk the amount of fat complexed at a given solids-not-fat level reaches a limiting value, but in the 4:1 concentrated milk a maximum value for the formation of the complex was not reached in the range of fat content examined. There is either a factor limiting complex formation at a 1:1 concentration or a factor facilitating complex formation at a 4:1 concentration. This factor must be associated with the solids-not-fat content rather than the fat content, since in the 1:1 concentration a limiting value for complex formation is reached which does not occur at the 4:1 concentration.

In Figure 5 the fraction of the fat complex per gram of sedimenting nitrogen is plotted as a function of the free fat. By free fat is meant that fat which is not sedimented. In the accepted sense the free ligand concentration is an equilibrium concentration, i.e., it exerts a pressure on the bound ligand to maintain a constant distribution of ligand between free and bound states. The free fat plotted in Figure 5 is not an equilibrium fat at the time of analysis, but may have been such at the instant of homogenization. Assuming that it

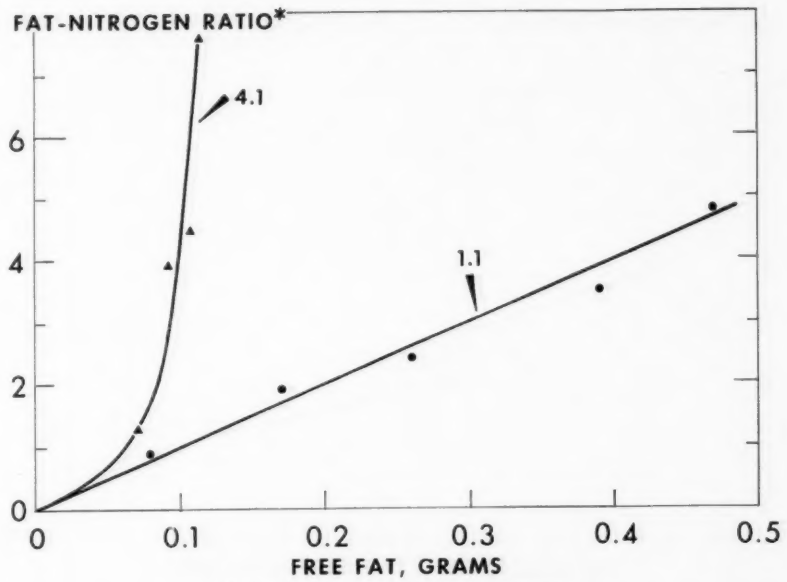

FIG. 5. The binding of fat by nonconcentrated and concentrated milks homogenized at 8,000 p.s.i.

does represent an equilibrium fat, the data of Figure 5 indicate that at a 1:1 concentration the fat partitions between the complex and the fat phase. This represents a statistical type of binding in which the amount of fat bound is proportional to the amount already bound, i.e., to the number of binding sites available. Since casein is the binding moiety a different interpretation may be made of the statistical concept. The distribution of sizes of caseinate micelles may be substituted for the distribution of the number of binding sites available. Whether this size distribution is continuous, as reported by Nichols *et al.* (5), or discrete, as reported by Ford and Ramsdall (3), the binding curve would in either case illustrate a partition effect when the size of the caseinate micelles is the only interaction parameter.

The curve for the fat complexed at a 4:1 concentration illustrates a cooperative kind of binding, in which the more fat that is bound, the easier it is for additional fat to be bound.

Another difference in the method of complexing of fat by 1:1 milk as compared with a 4:1 concentrate of the same milk is illustrated in Figure 6. To obtain the data of Figure 6, sucrose in increasing concentrations was added to aliquots of the 1:1 milk and to aliquots of the reconstituted concentrate. These solutions were centrifuged at increasing times proportional to their increase in viscosity; fat and nitrogen analyses were made of the sediments and the specific gravity of the supernatant liquids determined. The sedimenting complex of the reconstituted 4:1 concentrate underwent flotation at a specific

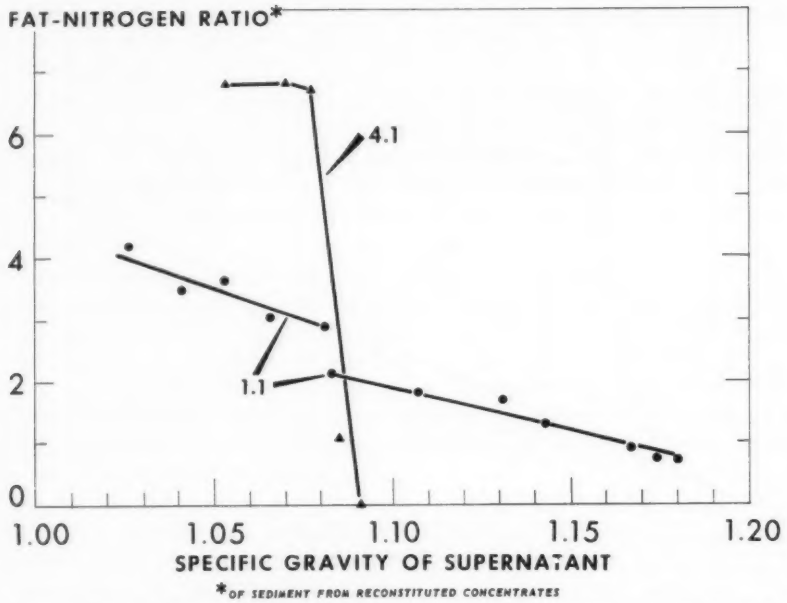

FIG. 6. Effect of concentration on specific gravity of fat-protein complex formed by homogenization. Homogenization pressure, 8,000 p.s.i. The 4:1 concentrate was reconstituted to 12.4% T.S. before adjustment of specific gravity with sucrose.

gravity of between 1.077 and 1.085 (20° C.). This narrow range of specific gravity of the complex indicates that only one species of complex forms when a 4:1 concentrate is homogenized at 8,000 p.s.i. In contrast, the fat-protein complexes formed in 1:1 milk homogenized at 8,000 p.s.i. have a range of different F/N ratios.

Effect of calcium on the formation of the fat-protein complex. It is usually necessary to consider the behavior of casein in terms of the amount of calcium in the system. To determine the effect of calcium on the formation of the fat-protein complex, different amounts of calcium as $CaCl_2 \cdot 2H_2O$ were added to aliquots of pasteurized milk (145° F. for 30 min.) and the milk allowed to equilibrate for 24 hr. at 36° F. The different samples of milk were warmed to 110° F. and homogenized at 8,000 p.s.i. The results are presented graphically in Figure 7. The addition of as little as 2.5 m*M* of calcium ions causes an increase in the amount of fat complexed; between 10 and 20 m*M* of calcium is sufficient to complex 100% of the fat. When the specific gravity of the solution containing 10 m*M* of added calcium was increased to 1.07 the fat-protein complex abruptly underwent flotation. The amount of fat complexed and the limited specific gravity range of the complex formed indicate that calcium causes formation of a fat-protein complex similar to that formed in a 4:1 concentrated milk.

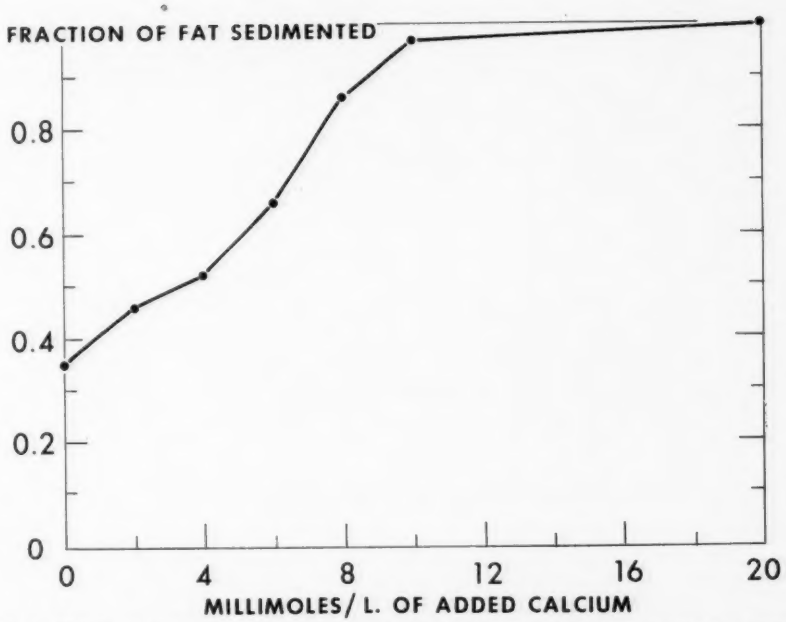

FIG. 7. Effect of increasing calcium ion concentration on formation of fat-protein complex.

DISCUSSION

It has been demonstrated that milk fat and the caseinate micelles of milk interact when homogenized. From the data obtained, certain speculations may be made about the mode of interaction. These speculations concern the effect of homogenizing upon the caseinate micelles, the effect of concentration upon the caseinate micelles, and the structure of the fat-caseinate complex.

The fat-protein interaction could not be induced by separately homogenizing the skimmilk and the fat and then mixing them. The chemistry of fat suggests that the only effect of homogenization on it should be increased surface area and, thereby, increased surface energy. If increase in surface energy or decrease in size were the only requirements for the interaction, it should occur when the fat homogenate is mixed with the skimmilk. That an interaction does not occur except at the homogenizing valve must mean that caseinate micelles are, in some manner, activated for the interaction by homogenizing.

In another experiment, mineral oil was complexed with the caseinate micelles by homogenization. Hydrocarbons should adsorb to the caseinate micelles only on a hydrocarbon site. Such hydrophobic sites, to be consistent with the accepted structure of proteins in aqueous solution, would be within the micelles. Therefore, homogenizing must distort or open up the caseinate micelle. If the hydrocarbon portion were on the surface of the micelle, the amount of fat bound would be proportional to the micelle surface area. Assuming a sphere,

the fat bound would be proportional to the square of the micelle diameter, or L^2. The nitrogen content would be proportional to the volume, or L^3. The fat-nitrogen ratio would be L^2/L^3 $(=1/L)$ or, the smaller the micelle, the more fat would be complexed to a given amount of casein. But, in the experiment in which calcium was added to milk to determine its effect on complex formation, the amount of fat complexing (and the F/N) increased with micelle size, and not the converse. Therefore, the part of the micelle to which the fat is bound must not be on its surface.

A construct which describes the increase in F/N ratio with increase in micelle size is one having the caseinate micelle distorted into a shell about the fat. In this case, the fat complexed is proportional to the volume of the shell and the nitrogen is proportional to its area, or L^2. The F/N ratio becomes L^3/L^2 $(=L)$ and increases with micelle size. A variation in the F/N ratio would also be consistent with the data for the F/N ratio in Figure 6, since a distribution in size of caseinate micelles occurs in normal milk (3, 5).

The extent of fat-protein interaction in 4:1 concentrated milk, as compared with that in the same milk not concentrated, implies that concentration causes changes in the micelle structure. The concentration of milk fat produces no known changes in its physical and chemical properties. The ratio of total solids to fat is the same in the 4:1 concentrate as in the 1:1 milk. If the increased interaction between fat and casein at a 4:1 concentration resulted from closer proximity of the fat and casein during homogenization, the interaction should increase linearly with concentration. This does not occur. Instead, the concentration effect is critical in behavior, which implies a change in the physical and/or chemical properties of the caseinate micelle. This change is manifested in two ways: (1) a large increase in the amount of fat bound, and (2) the fat-protein complex formed becomes essentially of one discrete size. If the interpretation is correct, that the amount of fat bound is a function of micelle size, only one size of caseinate micelle exists at a concentration of 37% T.S. and above.

REFERENCES

(1) Association of Official Agricultural Chemists. Official Methods of Analysis. 6th ed. Washington, D. C. 1945.

(2) Brunner, J. R., Duncan, C. W., and Trout, G. M. The Fat-Globule Membrane of Nonhomogenized and Homogenized Milk. Food Research, 18: 454. 1953.

(3) Ford, T. F., and Ramsdell, G. A. The Colloidal Proteins of Milk. Proc. XIIth Intern. Dairy Congr., (Stockholm), 2, 17. 1949.

(4) Ford, T. F., Ramsdell, G. A., Landsman, Shirley G., and Alexander, T. G. Nitrogen Factor for the Calcium Caseinate–Calcium Phosphate Complex in Milk. J. Dairy Sci., 40: 1395. 1957.

(5) Nichols, J. B., Bailey, E. D., Holm, G. E., Greenbank, G. R., and Deysher, E. F. The Effect of Preheating on the Dispersity of Calcium Caseinate in Skim Milk. J. Phys. Chem., 35: 1303. 1931.

(6) Palmer, L. S. The Structure and Properties of the Natural Fat Globule "Membrane". A Historical Review with Experiments Bearing on a Physico-Chemical Explanation. J. Dairy Sci., 27: 471. 1944.

(7) Sinnamon, H. I., Aceto, N. C., Eskew, R. K., and Schoppet, E. F. Dry Whole Milk. I. A New Physical Form. J. Dairy Sci., 40: 1036. 1957.

THE SOLUBILITY IN AQUEOUS UREA SOLUTIONS OF THE MICELLAR CASEINATES OF MILK AND MILK PRODUCTS SUBJECTED TO VARIOUS STERILIZING HEAT TREATMENTS

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SUMMARY

A study was made of the changes that occur in the micellar caseinates when heated in their natural environment.

The caseinates were separated from heated milks by centrifugation at 27,000 times gravity in a Servall centrifuge, followed by washing and lyophilizing. The dry caseinates were equilibrated by shaking for 24 hr. at 25° C. in 6.6 *M* urea solution. After dilution to 3.3 *M* urea, and equilibration for 48 hr., the solutions were centrifuged to separate the insoluble caseinates. The soluble caseinates of the solution were determined by micro-Kjeldahl analyses after dialysis to remove urea. Solubility curves, obtained by plotting the amount of the casein in 3.3 *M* urea solution against the amount of micellar caseinates present in the system, exhibit solubility phenomena characteristic of heterogeneous systems and show that:

1. Increased heating decreases the solubility of the caseinates in aqueous urea.
2. Fat present in milk during heating decreases the solubility in urea.
3. Increased solids content of milk gives decreased protein solubility in aqueous urea after heating.
4. High-temperature—short-time methods of sterilizing concentrated milk products generally produce less change in the protein solubility in urea than does the conventional sterilization method.
5. The presence of citrate and phosphate ions in the urea system increases the protein solubility.
6. The heat-produced protein component in the casein micelle concentrates in the 3.3 *M* urea insoluble fraction.

Heat sterilization produces a number of undesirable organoleptic and physical changes in milk products (10). The mechanism of the chemical reactions underlying these gross changes is not well understood, but many of the observed changes are thought to be associated with heat-induced modification of the milk proteins. Study of the milk proteins and the changes they undergo during heating is, therefore, of importance in the development of superior dairy products.

This paper reports results obtained during a study of the physical and chemical changes that occur in the casein fraction of milk and milk products during sterilizing heat treatments.

Casein (8), which is the principal protein fraction of milk (2), has been shown to exist in the forms of large micelles of complex composition (7). Various methods have been devised to isolate and fractionate these micellar proteins (4, 5, 11, 12). Up to the present at least five different caseins have been found in the casein micelle, in addition to inorganic ions and colloidal salts.

Experimental work using alpha and beta caseins, two of the more important constituents of the micelle, has been interpreted to indicate that these caseins

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have no specific three-dimensional space configuration and can, therefore, be considered to act as denatured globular proteins (3). If this is true, then the caseins should not be denatured by high heat treatment in neutral solutions. However, other work (6, 13, 14) has shown that heat does change the physical and chemical properties of the caseins isolated from milk. The changes were reported to be both reversible and irreversible, depending on the severity of the heat treatment employed. Since this earlier work was carried out in simplified model systems, it was considered desirable to study the changes that occur in the micellar proteins of milk when heated in their native environment, wherein the possibility of reaction with the other constituents of milk exists. Previously reported work (9) had shown that an electrophoretically unique protein (X), which had a mobility of 6.0×10^{-5} cm.^{2} volt^{-1} sec.$^{-1}$, appears in the casein micelle of heat-sterilized milks. To facilitate the eventual isolation of this protein, a solubility study of the micellar proteins isolated from such heated milks by centrifugation was made. Solutions of 3.3 M urea were used to determine maximum differences in solubilities of the caseinates. This concentration of urea produces a median solubility between insolubility of these caseinates in water and complete solubility in 6.6 M urea solutions.

EXPERIMENTAL PROCEDURE

Preparation of material. The micellar casein complex used for the determination of solubility was sedimented from the appropriate milk product by centrifuging for 1 hr. in a Servall super-speed angle-head centrifuge at a speed of about 14,000 r.p.m. and a centrifugal force of approximately 27,000 times gravity. The caseinate complex was washed by redispersing in distilled water, using a mortar and pestle or Waring Blendor. After two washings the redispersed caseinate was hard-frozen in metal trays, and the material was lyophilized in a pilot plant model of a Stokes shelf dryer. The dried complex was ground to a fine powder using a mortar and pestle. It should be noted that even at the low centrifugal force used in this preparation there is the possibility of the inclusion of small amounts of heat-aggregated whey proteins with the sedimented caseinates.

Determination of solubilities. In each series of solubility experiments, sufficient of the dried caseinate complex was placed in a 50-ml. plastic centrifuge tube and enough water added to make 15 ml. of solution containing 2, 4, 6, 8, 10, 12, 14, and 18 g. per 100 ml. Sufficient urea had previously been added to make the solution 6.6 M when made up to the 15-ml. mark. The tubes were shaken for 24 hr. at 25° C., diluted with water to the 30-ml. mark to bring the urea concentration to 3.3 M, and shaken for an additional 48 hr. to insure equilibration. The pH of the solutions was 7.3 ± 0.2. The tubes were then centrifuged in the Servall centrifuge for 1 hr. at 14,000 r.p.m. Aliquots of the clear solutions containing the dissolved caseinates were placed in small dialysis sacks and the contents dialyzed against distilled water until no urea was shown to be present. The aqueous contents were pervaporated, the dried

sack and contents digested, and nitrogen and protein determined by the conventional Kjeldahl micro-method.

The distribution of the several components of the original caseinate complex in that portion dissolved by the 3.3 *M* urea, and in the portion undissolved by urea, was determined by taking aliquots of the above clear liquid and of the sedimented and undissolved portion and dialyzing to equilibrium against veronal buffer containing 0.05 *M* NaCl at pH of 8.4 and ionic strength of 0.1. After clearing the dialyzed solution by centrifugation, an electrophoretic analysis was conducted in a portable Aminco apparatus, using a standard 8-ml. analytical cell. The photographic patterns were projected and traced on graph paper. Protein distribution and mobilities were measured, using conventional techniques (1).

The effect of 0.1 *M* citrate and of 0.1 *M* phosphate on the solubility of the micellar caseinates centrifuged out of heated skimmilk was studied in a similar manner.

RESULTS

The solubility curves obtained by plotting the amount of soluble casein against the amount of micellar caseinate present in the system have forms characteristic of heterogeneous systems and are shown in Figures 1-4. A comparison of these curves shows the effects of various processing and chemical factors on the solubilities of the caseinate micelles.

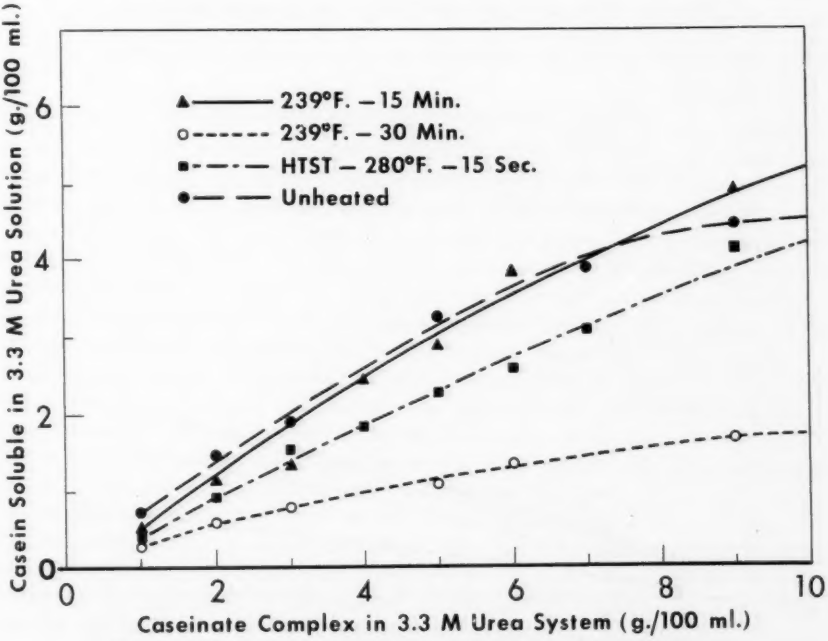

FIG. 1. Effect of heat treatment of milk on caseinate solubilities.

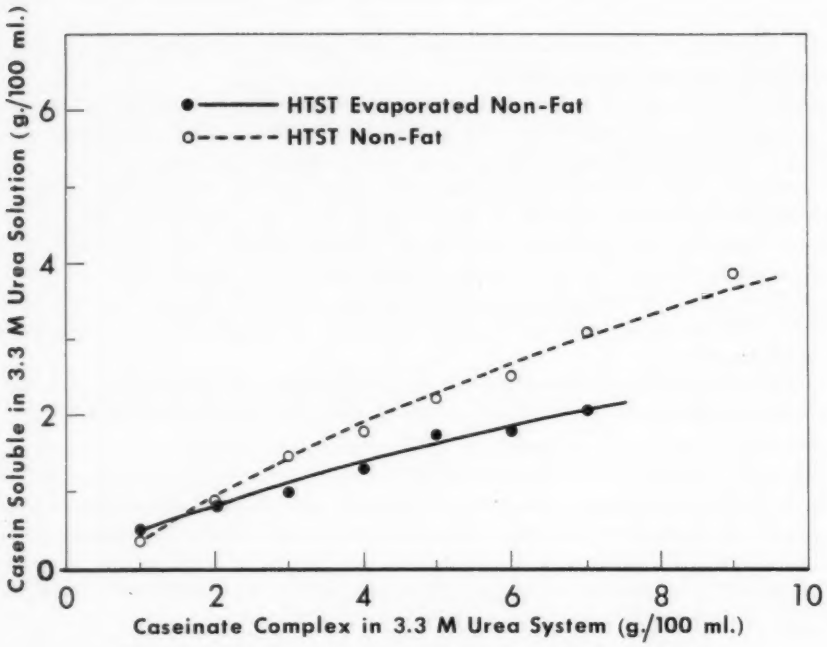

FIG. 2. Effect of solids concentration on caseinate solubilities.

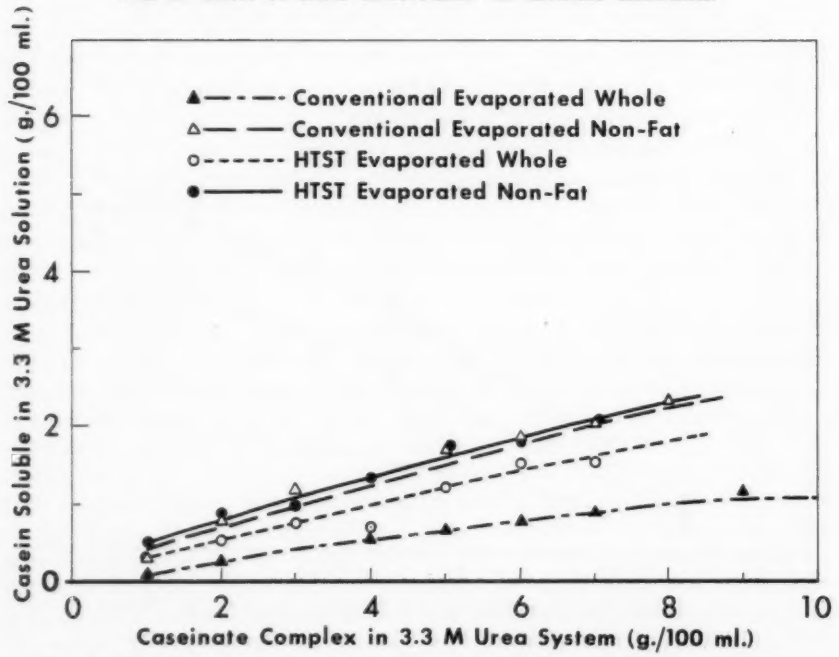

FIG. 3. Effect of fat content and of sterilizing methods on caseinate solubilities.

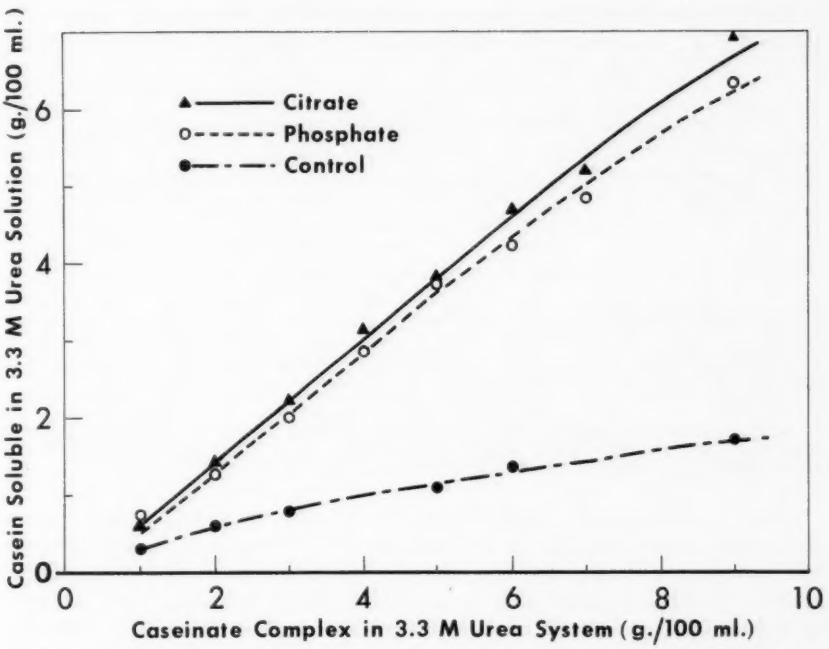

FIG. 4. Effect of phosphates and citrates on caseinate solubilities. Milk at 239° C.—30 min.

The effect of intensity and amount of heat treatment on the solubilities of caseinates from normal strength skimmilk is shown in Figure 1. High heat treatment produces caseinates of low solubility, H.T.S.T. (280° F. for 15 sec.) treatment produces those of intermediate solubility, and the caseinates subjected to low heat treatment are practically as soluble as those from unheated skimmilk. The effect of solids concentration on the solubility of caseinates in urea solutions is shown in Figure 2. High-temperature—short time heat treatment on single-strength nonfat milk produces caseinates of higher solubility than does the same heat treatment on double-strength evaporated nonfat milk.

A comparison of the two conventional evaporated milk curves in Figure 3 shows that the presence of fat during the conventional process of heating of 2:1 evaporated milk decreases the solubility of the caseinates. Similarly, the H.T.S.T. curves on this same figure show the same results. By comparing the two H.T.S.T. evaporated curves with the two conventional evaporated curves, the effect of the method of sterilization of evaporated milk on the solubility of the caseinates is shown. It is evident that the H.T.S.T. method of sterilization has less effect on the solubility of the caseinate in urea than does the conventional method, especially in the case of the evaporated whole milk. The difference in the effect of the two methods of sterilization is less apparent for the evaporated nonfat milks.

The increase in solubility in urea solution of the caseinates from severely heated milk in the presence of citrate and of phosphate ions is shown in Figure 4.

TABLE 1

Electrophoretic distribution of proteins in 3.3 molar urea systems

Type of milk and heating	Phase							
	Soluble				Insoluble			
	α Casein	X	β Casein	γ Casein	α Casein	X	β Casein	γ Casein
	(%)							
H.T.S.T. evaporated whole	53.4	8.9	28.3	8.9	95.0	5.0	0.0	0.0
H.T.S.T. evaporated skim	45.8	14.7	30.1	9.4	55.3	44.7	0.0	0.0
Conventional evaporated whole[a]	39.5	35.3	25.2	0.0	100.0	0.0	0.0	0.0
H.T.S.T. Skimmilk	72.5	5.5	22.0	0.0	83.5	10.1	6.4	0.0
Skim—115° C. 30 min.	59.0	11.0	17.3	12.7	69.0	22.0	8.9	0.0
Skim—115° C. 15 min.	66.6	11.6	18.8	3.0	75.0	8.6	16.4	0.0

[a] Very little of the insoluble phase was capable of being solubilized by veronal buffer in preparation for electrophoretic analysis and, therefore, the percentages given probably do not represent a true distribution of the various components.

Table 1 shows some typical results on the electrophoretic distribution of the various casein components between the 3.3 *M* urea soluble and insoluble fractions. In connection with the possibility of the inclusion of heat-coagulated proteins mentioned previously, it should be noted that the various casein fractions may contain some of these whey proteins, even though the mobilities actually found are normal for the casein fractions indicated. The beta casein and gamma casein components have a disposition to concentrate in the urea-soluble fraction while alpha casein and the heat-produced *X* component have a tendency to concentrate in the urea-insoluble fraction. This tendency may make it possible for the *X* component to be separated from the other components by convection electrophoresis or other methods of purification, so that its identity and composition may be studied.

ACKNOWLEDGMENT

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REFERENCES

(1) Alberty, Robert A. An Introduction to Electrophoresis. Part I: Methods and Calculations. J. Chem. Ed., 25: 426. 1948.

(2) Davies, W. L. The Chemistry of Milk. Chapman and Hall, Ltd., London. p. 7. 1939.

(3) Halwer, Murray. Light Scattering Study of Effect of Electrolytes on α- and β-Casein Solutions. Arch. Biochem. and Biophys., 51: 79. 1954.

(4) Hipp, N. J., Groves, M. L., Custer, J. H., and McMeekin, T. L. Separation of γ-Casein. J. Am. Chem. Soc., 72: 4928. 1950.

(5) Hipp, N. J., Groves, M. L., Custer, J. H., and McMeekin, T. L. Separation of α-, β- and γ-Casein. J. Dairy Sci., 35: 272. 1952.

[1] Deceased.

(6) HOWAT, G. R., AND WRIGHT, N. C. The Heat-Coagulation of Caseinogen. I. The Role of Phosphorus Cleavage. Biochem. J., 28: 1336. 1934.

(7) MCMEEKIN, T. L., "Milk Proteins" in "The Proteins" edited by Neurath, H., and Bailey, K. Vol. II, Part A, p. 400. Academic Press, New York. 1954.

(8) MULDER, G. J. Zusammensetzung von Fibrin, Albumin, Leimzucker, Leucin u.s.w. Ann. der Pharm., 28: 73. 1838.

(9) PALLANSCH, M. J., HALLER, H. S., AND MORIN, A. G. Physical and Chemical Properties of Some High Molecular Weight Complexes Isolated from Heated Skimmilk. J. Dairy Sci., 39: 911. 1956.

(10) PATTON, STUART. Browning and Associated Changes in Milk and Its Products: A Review. J. Dairy Sci., 38: 457. 1955.

(11) WARNER, R. C., AND POLIS, E. On the Presence of a Proteolytic Enzyme in Casein. J. Am. Chem. Soc., 67: 529. 1945.

(12) WAUGH, DAVID F., AND VON HIPPEL, PETER H. κ-Casein and the Stabilization of Casein Micelles. J. Am. Chem. Soc., 78: 4576. 1956.

(13) ZITTLE, C. A., AND DELLAMONICA, E. S. Viscosity and Flocculation of Heated β-Lactoglobulin Solutions: Effect of Calcium Concentration and pH. J. Dairy Sci., 39: 514. 1956.

(14) ZITTLE, C. A., DELLAMONICA, E. S., AND CUSTER, J. H. Effect of Certain Salts on Precipitation of Casein by Calcium Chloride and Heat. J. Dairy Sci., 40: 280. 1957.

ANALYSIS FOR CITRIC ACID IN PRESENCE OF CASEIN

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SUMMARY

Factors affecting citric acid determination in the presence of casein were studied with the pyridine–acetic anhydride method. Although the opalescence in skimmilk tests affected results by only 2%, the turbidity introduced by solutions of isolated casein could cause gross overestimation. A pH above 12.3 in the test aliquot assured a clear reaction mixture with both skimmilk and casein solutions. It is, therefore, recommended that test aliquots containing casein should comprise sufficient alkali to insure a clear reaction.

Acid casein required seven resuspensions in fresh washing solution at pH 4.5 for complete removal of occluded citrate, lactose, and calcium. Adsorption of citric acid by casein precipitated at the iso-electric point, or with trichloroacetic acid, was demonstrated and appears to account for the lower citrate concentration in acid sera, as compared to skimmilk. These results extend the conclusions of a previous paper.

In a previous publication (7) the authors recommended determination of citric acid in milk without prior separation of the casein. Results of direct analysis were 5 to 10% higher than values obtained with either trichloroacetic or hydrochloric acid sera. This discrepancy was attributed to adsorption of citric acid by the precipitated protein, a suggestion which had been made by Pucher *et al.* (12), who had observed a similar result (with a different citrate method) when albumin was precipitated from urine samples. The present authors' original conclusion (7) was that: "Attempts to detect interference of proteins have indicated that they do not interfere significantly. . . . We are therefore convinced that the higher values obtained by direct analysis of milk represent its true citric acid content within 2.5%."

In a private communication, Davies (1) reported that most samples of skimmilk gave a slight opalescence when analyzed by the direct method (7), and wondered whether this did not produce significantly high results; also, he doubted that acid-precipitated casein could bind citrate electrostatically. In a recent publication, Evenhuis (2) concluded that the direct method (7) gives erroneously high results, and that adsorption of citric acid by precipitated protein is unlikely.

The present paper shows that the slight opalescence obtained in direct analysis of skimmilk does not cause significant error but can, nevertheless, be completely eliminated. It is also shown that re-solubilized casein does not interfere with the determination of citric acid, provided sufficient alkali is added to prevent opalescence. However, casein preparations can introduce traces of citrate because of incomplete washing. Adsorption of citric acid by casein is demonstrated, extending the results of a study by Van Slyke (16, 17).

MATERIALS AND METHODS

Bulk skimmilk and skimmilk from individual cows were used in the study. Casein was prepared in bulk according to Van Slyke and Baker (15), or by

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adjusting 100-ml. aliquots of skimmilk to pH 4.5–4.6 with hydrochloric acid, then filtering through Whatman No. 2 paper. Casein was also prepared by adding 25 ml. of 60% w/v trichloroacetic acid (12% final concentration) to 100 ml. of milk (14).

Calcium was estimated turbidimetrically (6), lactose was determined colorimetrically (8), and citric acid was analyzed by the pyridine–acetic anhydride method (7, 9). The pH was measured with a Beckman Model G pH meter.

EXPERIMENTAL PROCEDURE AND RESULTS

Prevention of opalescence in the presence of casein. Opalescence was observed in some samples after direct analysis of citric acid in aqueous dilutions of skimmilk. Since this phenomenon had not been observed in previous studies with 1% solutions of casein in 0.1 *N* sodium hydroxide, it appeared probable that opalescence could be prevented by introducing alkali with the test solution. In a preliminary study with the citrate test at 32° C. (7) it was found that up to 0.5 meq. of NaOH could be tolerated per tube;[1] higher concentrations resulted in a marked reduction of color.

The possible effect of added alkali on the determination of citric acid in the presence of casein was studied with 1 and 3% w/v solutions of casein [Van Slyke and Baker (15)]. The casein was dissolved in various concentrations of sodium hydroxide up to 0.40 *N*, and 1.0-ml. aliquots were analyzed. Consistent results and clear reaction mixtures were obtained when the test aliquot was above pH 12.3 (Table 1); a lower pH in the aliquot led to increased turbidity in the test solution, with citrate recoveries as high at 175%. The yellow color of the reaction developed, even though citrate was not added to the casein solutions, indicating that the casein was contaminated with citric acid (see later).

A parallel study with bulk skimmilk (diluted 20/100) was also made, in which 0.50-ml. aliquots of the dilution were added per test, along with 0.50 ml. of sodium hydroxide solutions of various normalities up to 0.40 *N*. The desirable amount of alkali was introduced into the colorimeter tube as a separate addition,[2] to avoid loss of citrate by co-precipitation with calcium phosphate (13). The opalescence obtained at low additions of alkali in skimmilk (Table 1) had only a slight effect on determination of citric acid; however, a clear test could be obtained by adding 0.2 meq. of NaOH per test. The effectiveness of NaOH was confirmed with three other samples of skimmilk (diluted 20/100) from different sources. Analysis by the original citrate method (7) gave values (μg/ml) of 1,449, 1,441, and 1,213, compared with (respectively) 1,423, 1,407, and 1,185 in the presence of 0.2 meq. of NaOH/test; the difference was, therefore, 1.8, 2.4, and 2.4%, respectively. However, while the original method re-

[1] With aliquots containing 1% v/v chloroform as a bactericide, a red color can form in the presence of NaOH and pyridine (5) but disappears on adding the acetic anhydride, with no adverse effect on the subsequent citrate reaction.

[2] In adding the NaOH directly into the colorimeter tube, the aqueous phase in the test must be kept constant, since water is one of the reactants (7). The size of the skimmilk aliquot must, therefore, be reduced by an equivalent volume.

TABLE 1

Effect of alkali on citric acid test with casein solutions and diluted skimmilk

Type of aliquot[a]	NaOH added, meq/test	pH of aliquot	After citrate analysis		
			Citric Acid		Appearance of test solution
			Measured ($\mu g/ml$)	Recovery (%)[b]	
1% Casein	0.015	10.79	17.50	177.5	Turbid
1% Casein	0.025	11.33	12.34	125.2	Turbid
1% Casein	0.050	11.91	11.77	119.4	Opalescent
1% Casein	0.075	12.16	9.73	98.7	Clear
1% Casein	0.10	12.30	9.86	100.0	Clear
1% Casein	0.20	12.40	10.00	101.4	Clear
1% Casein	0.30	12.40	9.59	97.3	Clear
1% Casein	0.40	12.40	9.86	100.0	Clear
3% Casein	0.025	8.38	53.69	175.6	Turbid
3% Casein	0.050	11.10	38.25	125.1	Turbid
3% Casein	0.075	11.93	36.98	121.0	Opalescent
3% Casein	0.10	12.18	32.43	106.1	Opalescent
3% Casein	9.20	12.40	30.57	100.0	Clear
3% Casein	0.30	12.38	30.23	98.9	Clear
3% Casein	0.40	12.39	30.57	100.0	Clear
Skimmilk	0	7.02	1751	101.2	Opalescent
Skimmilk	0.050	12.02	1720	99.4	Opalescent
Skimmilk	0.10	12.28	1694	97.9	Opalescent
Skimmilk	0.20	12.37	1731	100.0	Clear

[a] 1.0 ml. of the alkaline casein solutions was taken for analysis; 0.50 ml. skimmilk (20/100) + 0.50 ml. NaOH were taken for analysis.
[b] Recovery is based on the last sample in each group.

sulted in opalescent solutions after citric acid analysis, the modification with 0.2 meq. of NaOH gave clear reaction mixtures throughout.

Washing of casein. Formation of yellow color with casein solutions, especially in the absence of turbidity (Table 1), suggested that triple-washed casein contained citric acid. A study of the effect of washing on the removal of citric acid from casein was, therefore, undertaken. Acid casein, obtained from 100-ml. portions of skimmilk by adding hydrochloric acid, was dispersed in 100 ml. of approximately 0.1 *M* sodium acetate buffer solution at pH 4.50. The mixture was thoroughly suspended, then refiltered within approximately 10 min.; in this manner, acid caseins resuspended from zero to nine times, along with the corresponding acetate washing solutions, were obtained. Each sample of casein was then dispersed in approximately 50 ml. of water, 3.0 ml. of 10.0 *N* NaOH added, and the volume adjusted to 100 ml. Results of citric acid analysis (Figure 1-A) showed that citrate-free casein was obtained after seven resuspensions in equivalent volumes of acetate buffer; the thoroughly washed casein showed no interference with the citrate method. Throughout, the concentration of citric acid in the casein solution was systematically related to that of the corresponding acetate washing solution, indicating that the resuspension technique was fully adequate for the establishment of equilibrium. Therefore, the concentration found in the acetate buffer reflected the degree of casein purity.

In a duplicate experiment with another lot of milk, the acetate washing

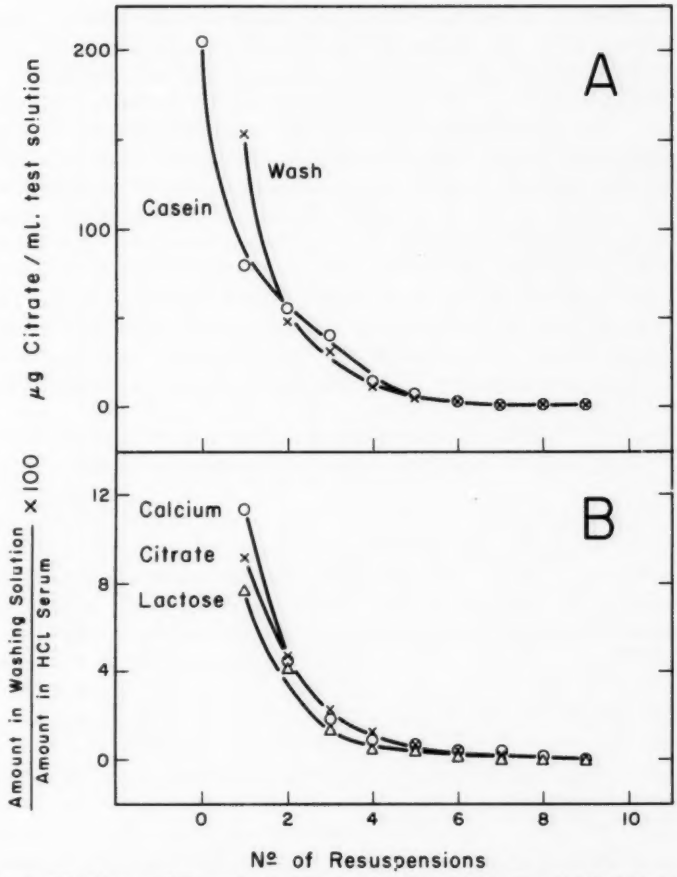

FIG. 1. Washing of HCl casein in 0.1 *M* sodium acetate buffer at pH 4.50 (casein from 100 ml. milk resuspended in 100-ml. volumes of fresh washing solution).

A: Concentration of citric acid in the washing solution, viz., that remaining in the casein (dissolved in an equal volume of 0.3 *N* NaOH).

B: Removal of various milk components from casein, expressed as a percentage of the initial serum concentration.

solutions were analyzed for lactose and calcium, in addition to citric acid (the casein was not analyzed). As in the previous experiment, at least seven resuspensions were required before removal of any of the three components could be considered complete (Figure 1-B). The results, expressed as per cent of the original acid serum concentration, show that retention by (and subsequent removal from) casein is proportional to the initial concentration of the various components.

Adsorption of citric acid by casein at pH 4.5–4.6. Van Slyke and Van Slyke (16) have shown that acid-precipitated casein can retain various acids (i.e.,

hydrochloric, sulfuric, acetic, and lactic) by means of a Freundlich (charcoal-type) adsorption (17); the authors did not study citric acid. However, Freundlich's data (4) indicated that citric acid retention was to be expected.

To study adsorption, casein was prepared from 100-ml. lots of skimmilk by precipitation with hydrochloric acid or with trichloroacetic acid. The two casein samples were washed nine times by resuspension in 0.1 *M* acetate buffer at pH 4.50, then suspended in 100 ml. of approximately 9.5 m*M*/liter sodium citrate buffer solution at pH 4.58. After 30 min., during which the casein suspensions received periodic agitation, the total volume was noted, the suspensions were filtered, and the filtrates kept for citrate analysis. The caseins were dried in an air oven at 50° C. and their weight was used to correct the total volume.[3]

Suspension of a thoroughly washed casein (whether obtained by HCl or TCA precipitation) in citrate solution at the iso-electric point of casein resulted in a decrease of from 7 to 9% in the citric acid concentration (Table 2).

TABLE 2

Adsorption of citric acid by casein at the iso-electric point

Type of sample	Total volume of suspension	Casein		Net volume	Citric Acid, μg/ml		
		Wt.	Volume		Determined	Corrected for volume	Recovery (%)
	(ml.)	(g.)	(ml.)	(ml.)			
HCl casein filtrate	114.0	3.15	2.10	111.90	1,444	1,616	90.9
TCA casein filtrate	116.0	3.23	2.15	113.85	1,448	1,649	92.8
Citrate solution	.....	.....	.....	100.0	1,777	.....	100.0
Milk I HCl serum	.....	.....	.....	.....	1,579	1,576[a]	94.3
Milk I Skimmilk[b]	.....	.....	.....	.....	1,671	.....	100.0
Milk II HCl serum	.....	.....	.....	.....	1,623	1,620[a]	95.2
Milk II Skimmilk[b]	.....	.....	.....	.....	1,702	.....	100.0

[a] Includes corrections (× 1.018) for 3.0 *N* HCl addition and (× 0.98) for approximate volume occupied by casein.

[b] 0.2 meq. of NaOH were added per test.

For purposes of comparison, two samples of skimmilk and their hydrochloric acid sera also were analyzed; the results with hydrochloric acid sera were approximately 5% lower than those obtained with their respective skimmilks (Table 2).

DISCUSSION

The results show that a turbid or opalescent reaction mixture can occur in the citrate test if the aliquot of test solution is below pH 12.3. Waugh and von Hippel (18) have demonstrated that the dispersibility of a re-solubilized acid casein was improved by an alkaline treatment at pH 12.0. Our results indicate that opalescence does not introduce a large error in the analysis of diluted skimmilk (i.e., < 2.5%); however, it can cause overestimation at the

[3] The volume occupied by casein was estimated by suspending 3.0 g. of Van Slyke and Baker casein (15) in 20 ml. of the 9.5 m*M*/liter citrate solution; the total volume obtained was 22 ml., giving an apparent specific volume of 0.67, which approximates the values reported by Ford *et al.* (3).

higher casein levels introduced by aliquots of 1 and 3% solutions of re-solubilized casein (Table 1). It appears probable that lack of gross interference with skimmilk is due—at least in part—to improved dispersibility of the natural casein aggregate under conditions of the citrate test. With normal skimmilk, a clear test reaction is obtainable by addition of 0.2 meq. of NaOH/test; however, with abnormal and modified milks, the required amount of alkali should be determined by a preliminary test. With skimmilk, the desired amount of alkali must be added as a separate addition to the colorimeter tube, and the volume of aqueous phase kept constant by a corresponding decrease in the size of the skimmilk aliquot. Recently, Evenhuis (2) used sodium caseinate solutions mixed in various proportions with a known sodium citrate solution (pH. 6.8) in a study of the pyridine–acetic anhydride method. He also fermented the citrate from milk and found that the casein had to be redissolved by addition of alkali to pH 6.9. In both types of experiment, the pH of the test aliquots was obviously below the value of 12.3 required to assure a clear reaction mixture and, therefore, his conclusion that the effect observed with these re-solubilized casein solutions would apply to analysis of normal skimmilk is erroneous.

Complete removal of citric acid (and other milk components) from acid-precipitated casein requires very thorough washing (Figure 1-A and 1-B); this is particularly important where the effect of casein on a citrate method is being studied. The gradual removal of milk components by successive washing treatments of acid casein has also been noted by Muller (10), who performed lactose determinations on the casein as a means of assessing the efficiency of washing. Patten and Hergstrom (11) recommend agitation or compression of the curd in the presence of the acid whey to extrude calcium, after which the casein is triple-washed. Thoroughly washed casein (resuspension 7, 8, and 9 in Figure 1-A) showed no interference with the citrate method, even though 1.0 ml. of approximately 3% solution (in 0.3 *N* NaOH) was used in the citrate test. In contrast, the triple-washed Van Slyke and Baker casein (15) used in the casein:alkali study (Table 1) contained approximately 1.0 μg. of citric acid per milligram of casein. It is concluded that the substance identified as chromogenic material in casein in a previous publication (7) was, in fact, residual citric acid.

Citric acid is adsorbed at the iso-electric point by casein obtained either by HCl or by TCA precipitation (Table 2). Retention of citrate by casein is lower in the presence of milk serum than in solutions containing citrate only, probably because other negatively charged components of the serum are adsorbed and displace some citrate (16, 17). A previous study (7) had shown that the citrate content of HCl serum was identical to that of TCA serum. This indicated that the degree of citrate adsorption was similar in both systems, even though the over-all positive charge of the casein particles was much greater in the TCA-treated milk (pH approximately 1.0). Because of this adsorption phenomenon, analysis of HCl or TCA acid serum does not provide a true value for total citrate in skimmilk, but yields a result which is from 4 to 10% low.

REFERENCES

(1) DAVIES, D. T. Hannah Dairy Research Inst., Kirkhill, Ayr, Scotland. Private communication. October 16, 1959.

(2) EVENHUIS, N. Determination of Citric Acid in Milk. Netherlands Milk and Dairy J., 13: 250. 1959.

(3) FORD, T. F., RAMSDELL, G. A., AND ALEXANDER, T. G. Apparent Specific Volume of the Calcium Caseinate–Calcium Phosphate Complet in Milk. J. Dairy Sci., 42: 397. 1959.

(4) FREUNDLICH, H. Uber die Adsorption in Lösungen. Z. physik. Chem., 57: 385. 1907.

(5) FUJIWARA, K. New Reaction for the Detection of Chloroform. Chem. Abstr., 11: 3201. 1917.

(6) MARRIER, J. R., AND BOULET, M. Direct Microdetermination of Calcium in Milk. J. Agr. and Food Chem., 4: 720. 1956.

(7) MARIER, J. R., AND BOULET, M. Direct Determination of Citric Acid in Milk with an Improved Pyridine–Acetic Anhydride Method. J. Dairy Sci., 41: 1683. 1958.

(8) MARIER, J. R., AND BOULET, M. Direct Analysis of Lactose in Milk and Serum. J. Dairy Sci., 42: 1390. 1959.

(9) MARIER, J. R., AND BOULET, M. Preparation of a Standard for Citric Acid Analysis. J. Dairy Sci., 42: 1885. 1959.

(10) MULLER, L. L. Investigations in Casein Manufacture and Quality. Australian J. Dairy Technol., 14: 81. 1959.

(11) PATTEN, J. L., AND HERGSTROM, E. G. The Manufacture of Lactic, Hydrochloric, and Rennet Casein. Australian J. Dairy Technol., 10: 160. 1955.

(12) PUCHER, G. W., SHERMAN, C. C., AND VICKERY, H. B. A Method to Determine Small Amounts of Citric Acid in Biological Material. J. Biol. Chem., 113: 235. 1936.

(13) PYNE, G. T., AND MCGANN, T. C. A. Colloidal Phosphate of Milk. II. Influence of Citrate. J. Dairy Research, 27: 9. 1960.

(14) ROWLAND, S. J. The Determination of the Nitrogen Distribution in Milk. J. Dairy Research, 9: 42. 1938.

(15) VAN SLYKE, L. L., AND BAKER, J. C. The Preparation of Pure Casein. J. Biol. Chem., 35: 127. 1918.

(16) VAN SLYKE, L. L., AND VAN SLYKE, D. D. The Action of Dilute Acids upon Casein when no Soluble Compounds are Formed. Am. Chem. J., 38: 383. 1907.

(17) VAN SLYKE, L. L., AND VAN SLYKE, D. D. Adsorption of Acids by Casein. J. Biol. Chem., 4: 259. 1908.

(18) WAUGH, D. F., AND VON HIPPEL, P. H. k-Casein and the Stabilization of Casein Micelles. J. Am. Chem. Soc., 78: 4576. 1956.

COAGULATION OF MILK FOR CHEESE MAKING BY ESTER HYDROLYSIS[1]

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SUMMARY

The production of a coagulum in milk with compounds which hydrolyze slowly to produce acids has been investigated. Of the compounds tested (including anhydrides, esters, lactones, and lactides) D-glucono-delta-lactone and meso-lactide produced a uniform coagulum suitable for the manufacture of Cottage Cheese. Meso-lactide and D-glucono-delta-lactone at weights equal to 8.8 and 12.3% of the SNF, respectively, gave a pH of about 4.6 after complete hydrolysis. It was necessary to use about 0.02% calcium chloride with the lactide to obtain normal expression of the whey during cooking. The coagulation time varied with the temperature of the milk. Lactide required 2 hr. at 25° C. and 47 min. at 37.5° C. Glucono-lactone required about 15 hr. at 20° C. and 3.5 hr. at 40° C. At higher coagulation temperatures the curd was firm enough to cut at a higher pH than is normally used. The addition of rennet made it possible to cut the curd at pH's higher than normal and thus reduced the setting time. A coagulation temperature above 37° C. caused excessive matting of the curd. The Cottage Cheese produced by this method had a bland flavor and was very similar in appearance to that produced by starter culture.

One of the vital steps in the manufacture of many varieties of cheese is the development of lactic acid in the cheese milk by a lactic starter culture. This step is also the source of many of the problems of cheese making. The use of microorganisms for this purpose necessarily imposes certain limitations on the conditions that may be employed. The activity of the culture must be maintained, temperatures must be controlled during the process to obtain the desired rate of acid production, and the presence of inhibitory agents must be avoided. With all these precautions the product may lack uniformity, due to variations in the lactic culture used. In an effort to avoid these difficulties, various investigators (10, 12, 13) have attempted to bring about acidification and coagulation of milk by the direct addition of various acids to the milk. These early attempts were unsuccessful, since it was not possible to distribute the acid uniformly through the milk to produce a uniform coagulum or gel. The addition of small amounts of acid, insufficient to cause complete coagulation, resulted in undesirable localized areas of coagulation.

Hucker and Marquardt (3) reported the use of pure lactic acid in place of a lactic starter in the manufacture of Cheddar Cheese from raw milk. However, these workers also reported making Cheddar Cheese without either starter or lactic acid, and it would seem that their results were due to the initial flora of the raw milk rather than to the acidulation of the milk with lactic acid.

Mabbitt *et al.* (7) reported that glucono-delta-lactone, when dissolved in milk, hydrolyzed slowly to produce gluconic acid, and formed a suitable curd for making Cheddar Cheese. The use of this compound modified the manu-

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facturing process in two ways: (1) The rate of hydrolysis was most rapid immediately after the lactone was added, subsequently decreasing at a uniform rate. Therefore, during cooking, and afterward, little acid was produced in the curd particles if no lactic culture was used; (2) the resulting curd contained more lactose than that made with a lactic culture, which would probably affect the microflora that developed during the curing period.

This paper describes attempts to make Cottage Cheese without a lactic culture. As the acid-producing agent, or acidogen, D-glucono-delta-lactone as well as other compounds which hydrolyze to produce the desired acidity were used.

METHODS

Each compound tested as an acidogen was dissolved in milk at a concentration calculated to yield a final pH of approximately 4.5. The compound was stirred into the milk and the rate of solution and pH drop were observed. Acidogens which dissolved readily and which gave a sufficient drop in pH within 16 hr. were examined further. The level of acidogen needed to yield a coagulum then was determined, and those which gave a smooth coagulum were used to make Cottage Cheese on a laboratory scale.

With the exception of the lactides, all of the acidogens were obtained commercially. The D-glucono-delta-lactone was obtained from Chas. Pfizer and Co.

Racemic and meso-lactides of lactic acid were prepared by a method similar to Carothers *et al.* (2), as follows: Nine hundred grams of 85% lactic acid were placed in a 1-liter round-bottomed flask, heated with a heating mantle, and stirred with a magnetic stirrer until the temperature of the contents reached 120° C. The temperature was maintained at 120° C. for about 6 hr., to drive off all water of dilution. The flask was then fitted with a distilling head and a 1-liter round-bottomed receiver and heated to 140 to 150° C. for 12 to 13 hr. under the pressure attainable with a water pump. During this time the pressure fell to a value of 11 mm. After 10 hr. of heating the process was interrupted and all the material which had distilled was returned to the reaction vessel. Next, 9 g. of zinc oxide was added, and the lactide was distilled under about 5 mm. pressure. The temperature ranged widely during the distillation, as this step is essentially a depolymerization of the long linear polymers of lactic acid to its volatile cyclic dimer. The distillate weighed 574 g.

Since the starting lactic acid is a racemic mixture, the distillate consists of three possible isomeric lactides, DD, DL, and LL. The DD and LL will form a racemic mixture, but the DL will have different properties. The DL and racemic mixture should be formed in equal amounts. Only one form of lactide melting at about 128° C. has been isolated from racemic lactic acid (2, 4, 11). The DD and LL forms by themselves are reported to melt at 95° C. (5, 6), and a racemic mixture of these is reported to melt at 125° C. (6). It was possible to isolate lactides melting at 128 and 41° C. from the distillate described. Since the 128°-melting form is the racemic mixture, the low melting form must be the meso (DL) form.[2] The two forms were separated as follows:

[2] This is the reverse of the notation used in our abstract, J. Dairy Sci., 42: 901. 1959.

The distillate was dissolved in 330 ml. of chloroform at the boiling point and cooled to 3° C. The crystals were filtered off, washed with 150 ml. of chloroform, and dried. The crystals weighed 231 g. The filtrate was heated to 120° C. to drive off the chloroform, and after the residue had cooled it was dissolved in 330 ml. of ether. The solution was cooled to 3° C., and the lactide crystallized as a hard cake. This was broken up, ground in a mortar, and filtered. The crystals which weighed 212 g. were washed with 150 ml. of ether. The crystals obtained from the chloroform melted at 123 to 127° C. The crystals obtained from the ether were predominantly the meso-lactide. They were further purified by heating to boiling in 330 ml. of ether. Some 12 g. of the material did not dissolve. This was filtered off and found to be racemic lactide. The filtrate was cooled to 0° C. and filtered and 179 g. of material melting at 42 to 45° C. were obtained. On cooling the filtrate to −30° C., 19 g. more (melting at 41 to 42° C.) was recovered. The latter material was found to have a saponification equivalent 72.90 (calc. 72.0). The carbon and hydrogen analysis indicated 49.99% carbon and 5.61% hydrogen (calculated 50.00% C., 5.60% H). The meso-lactide should be protected from moisture and stored in a desiccator. The racemic-lactide, if pure, will survive exposure to high humidities.

The laboratory scale preparations of Cottage Cheese were performed as follows: Two kilograms of fresh skimmilk or reconstituted Grade A low-heat nfdm[3] of known solids content were placed in an aluminum pan 11 by 8 by 3½ in., held in a constant temperature bath. The amount of acidogen added was based on the solids content of the milk, which is directly related to the buffer capacity, and was calculated by the equation:

$$\text{Weight of acidogen} = K\ (\%\ \text{solids})\ (\text{weight of milk})$$

where K is an empirical constant related to the nature of the acidogen. It was necessary to determine the weights and percentages to about one part in 100 for satisfactory control of the pH. The desired amount of acidogen was stirred into the milk by hand or with a reciprocating mechanical stirrer. The pH was determined with a Beckman zeromatic pH meter. Stirring was terminated before the pH reached 5.2. The acid generation was allowed to continue undisturbed until a firm coagulum had formed. At cutting, the pH usually was about 4.7 to 4.8, but it varied with temperature and the characteristics of the acidogen. The coagulum was cut with ½- or ¼-in. cheese knives, made to fit the pan. The cooking procedure varied somewhat with the temperature of the set and the behavior of the curd but, in general, resembled that used in making Cottage Cheese with lactic starter. The final cooking temperature usually was 130 to 140° F. In some trials, $CaCl_2$ and/or rennet extract also was used. When used, 0.02 to 0.04% $CaCl_2$ was added prior to the addition of the acidogen. Rennet extract was diluted 1:1000 with distilled water and the diluted extract added at the rate of 1 to 2.5 ml/Kg of milk after the acidogen had dissolved.

Several lots of Cottage Cheese were prepared on a pilot plant scale, using approximately 200 to 250 lb. of milk in a 50-gal. cheese vat. In these experi-

[3] Consolidated Badger Company, Shawano, Wisconsin.

ments D-glucono-delta-lactone was used, since it is the only suitable acidogen that is available on a commercial scale. The procedure was essentially the same as that used on the laboratory scale.

The solids in the milk were determined by the Mojonnier procedure (8). The solids in the Cottage Cheese were determined by drying 4 to 5 g. of curd in an aluminum dish containing dry sand at 105-110° C., in an atmospheric oven for 24 hr.

SELECTION OF SUITABLE ACIDOGENS

An acidogen, to be suitable, should exhibit the following characteristics: (1) It must be nontoxic. (2) It must not react with the constituents of milk to form toxic products or reduce its nutritive value. (3) It must dissolve readily in milk. (4) It must produce acid at a reasonable rate. (5) It must not alter the properties of the curd obtained. (6) It must not impart a flavor to the finished cheese. (7) It should be inexpensive and readily available.

There are many compounds which react with water to produce an acid, but most of them fail to meet some of the requirements mentioned above. This investigation has been restricted to compounds believed to be reasonably nontoxic and commercially available or readily prepared.

Of the compounds tested, the following were found unsuitable because of their limited solubility: racemic lactide, succinic anhydride, diethyl sulfate, sulfuryl chloride, phosphorus oxychloride, phosphorus pentachloride, acetyl chloride, and glutaric anhydride. These compounds either did not disperse at all in the milk or reduced the pH below 5.2 before they were completely dissolved. The result was a very uneven coagulum or coagulation on the bottom of the milk only. Stirring below pH 5.2 was considered detrimental to good coagulation.

Of the compounds tested, the following were rejected for slow or undetected production of acid: sulfamic acid, metaphosphoric acid, D-galactono-gamma-lactone, and D-glucurono-gamma-lactone.

Acetic anhydride, beta-propiolactone, and glutaric anhydride were rejected for altering the properties of the coagulum. As mentioned above, glutaric anhydride also was of limited solubility. Acetic anhydride and beta-propiolactone both produced coagulums, but a pH considerably lower than normal was required to form a firm gel. It is believed that these reagents acylate the amino groups on the casein as well as form acid. This alters the isolectric point of the protein. This was further substantiated by performing a formol titration on the acetic anhydride–treated milk (14). It was found that the acetic anhydride treatment reduced the formol titration approximately one half. Alteration of the protein was also indicated by the difficulty experienced in cooking out the curd. Even after long cooking the curd was mushy and had a high moisture. Most of the flavor of acetic acid could be removed when the cooked curd was washed.

Two compounds were found that seem to meet all the criteria mentioned above. They are meso-lactide and D-glucono-delta-lactone. When added to milk these form lactyl-lactic acid and D-gluconic acid, respectively. The lactyl-lactic

acid is not further hydrolyzed in the pH range encountered (4). It is believed that both of these compounds are harmless and readily metabolized. There is no reason to believe that they react with other groups in the milk; at least, they do not alter the coagulation properties of the casein or affect the cooking properties of the curd. It has been found that calcium ion must be added to get good expression of the whey when meso-lactide is used, but this is believed to have a direct effect on the mineral balance of the system. Meso-lactide has the further disadvantage of not being very soluble in the milk; however, if it is well purified and finely divided it will work satisfactorily. It has the advantage of hydrolyzing very rapidly and shortening the coagulation time. D-glucono-delta-lactone is easily soluble in the milk and is commercially available; however, it is much slower in hydrolyzing than is lactide. Neither adversly affects the flavor of the cheese curd.

Other delta-lactones and lactides have not been tested, although there is no reason to think they might not work. Delta-lactones, in general, hydrolyze more rapidly than the gamma-lactones (9). The gamma-lactones tested here were all too slow. It has been reported that an enzyme catalyzing the hydrolysis of D-glucono-delta-lactone exists (1). This might be used to speed the hydrolysis. The only other lactide that is well known is glycolide, the lactide of glycolic acid. This has not been tried, but it is said to hydrolyze more rapidly than meso-lactide (4) and its solubility should not be much greater. If lactides were made from longer-chain alpha-hydroxy acids, it would be desirable to attach some hydrophilic group to the molecule to increase the water solubility of the resulting lactide.

COTTAGE CHEESE MAKING WITH ACIDOGENS

Data obtained in the manufacture of Cottage Cheese with various acidogens on a laboratory scale are given in Table 1. Table 2 presents the data obtained with D-glucono-delta-lactone on a pilot plant scale.

Table 1 shows that calcium chloride has a rather marked effect when meso-lactide is used, but does not affect the curd obtained with glucono-lactone. Thus, when lactide is used, the addition of calcium chloride markedly decreases the cooking time and facilitates the expression of the whey. Moreover, the level of lactide needed to achieve a given pH is decreased by the addition of calcium chloride. The effect of calcium chloride has not been studied systematically, but it appears that 0.02% restores the normal cooking properties of the curd. It seems probable that the lactyl-lactic acid formed ties up the calcium in the milk in some way.

Rennet added along with the acidogen decreases the coagulation time considerably. The resulting coagulum can be cut sooner and the acid will continue to develop after cooking has begun. This reduces the over-all time required.

The temperature at setting markedly affects the rate of hydrolysis of the acidogen. Glucono-lactone requires 900 to 1,000 min. to coagulate skimmilk at 20° C., but only one-fourth to one-fifth that length of time at 35° C. The hydrolysis of the lactide also is accelerated at higher temperatures. The higher temperature at coagulation with glucono-lactone is associated with a higher pH

at coagulation. Thus, a high setting temperature accelerates the cheese making process. Setting the milk at temperatures above 37° C., however, produces a coagulum that is very stringy and clings to the knives. The curd formed loses whey, and mats so rapidly that the individual cubes can not be kept separate. It does not appear possible to coagulate milk with an acidogen at temperatures above 37° C. and still follow normal Cottage Cheese making methods.

The final pH that will be reached is determined at the addition of the acidogen. Thus, contrary to usual practice, it is quite possible to let the coagulum set for long periods without fear that a higher acidity will develop. On the other hand, if the wrong amount of acidogen is added, the mistake can not be corrected. It has been found that the final pH can be controlled to within 0.1 pH unit if the solids and acidogen are known within 1% accuracy. Normal variations in the pH of fresh milk do not materially affect the level of acidogen required.

The titratable acidity is not a reliable measure of rate of acid development when acidogens are used. This is because the acidogen rapidly hydrolyzes under alkaline conditions. Thus, any attempt to titrate the milk to the phenolphthalein end point gives a measure more closely related to the total amount of acidogen than to the amount hydrolyzed. A pH meter is quite valuable in following the development of acidity. However, as the data indicate, the pH at which the curd was cut varies considerably, and the nature of the coagulum rather than the pH was used as a criterion for judging when the curd should be cut.

The experiments made on a laboratory scale, using the ½-in. knives, frequently resulted in nonuniform curd. An important part of this effect can be attributed to the pH meter. After the curd was cut, and before it was stirred, it was noted that the portion of the curd that had coagulated near the pH meter electrodes did not expel whey nearly as well as that further away. This is believed to be due to the effect of the potassium chloride from the calomel electrode on the salt balance of the milk.

The pilot plant experiments with glucono-lactone in general confirmed the laboratory scale tests. However, the whey was expelled more readily from the curd on a pilot plant scale because of the greater depth of curd.

The flavor of the Cottage Cheese prepared from meso-lactide and glucono-lactone was pleasant and bland. Creamed Cottage Cheese with a very mild flavor could be obtained by simply creaming the resulting curd. For a more flavorful Cottage Cheese, starter distillate could be added or the cream could be inoculated with a suitable lactic culture. The curd made with the glucono-lactone on a pilot plant scale seemed to absorb the creaming mixture more readily than curd made with lactic starter. There was some indication that Cottage Cheese prepared by the acidogen process is not as likely to become mealy if over-cooked, as is cheese produced with starter.

The results show that it is technically possible to prepare a very satisfactory Cottage Cheese by using meso-lactide or D-glucono-delta-lactone to develop acid in the milk. It is not clear to what extent this process might compete with the conventional procedure. It does not appear likely that the savings in time, labor,

TABLE 1

Laboratory scale production of Cottage Cheese

Acidogen	% Solids of milk	% $CaCl_2$	Rennet, (ml. 1:1000)	Amount, acidogen[a]	Coagulation temp. (° C.)	Coagulation time (min.)	Cutting pH	Knife size (in.)	Cooking time[c] (min.)	Cooking temp. (° C.)	Final pH	Curd Yield (%)	Curd Solids (%)	Remarks
Lactide[b]	9.00			0.098	26	120	4.69	¼	300	55	4.50	17.3	17.5	Hard to cook out
Lactide[b]	8.73		2	0.100	25	80	4.81	¼	440	60	4.59	16.0	20.2	Hard to cook out
Lactide	9.15		4	0.100	25	48	4.90	¼	310	55	4.50	19.0	18.2	Hard to cook out
Lactide	8.81	0.02		0.0885	25	150	4.71	¼	240	56	4.60	17.6	20.8	Normal curd
Lactide	8.65	0.02	4	0.100	25	45	5.00	¼	375	60	4.34	17.8	20.0	Too much acid, hard to cook out
Lactide	9.21	0.02	3	0.0885	25	120	4.75	¼	215	55	4.60	16.8	20.7	Normal curd
Lactide	8.90	0.02	3	0.0900	30.5	60	4.70	¼	180	55	4.47	15.8	20.9	Normal curd
Lactide	8.93			0.0875	30	120	4.71	½	240	55	4.58	16.8	20.2	Curd not uniform
Lactide	8.93	0.02		0.0875	37.5	47	4.68	½	233	57.5	4.60	17.3	19.2	Curd not uniform
Acetic anhydride	8.60	0.04		0.100	23	135	4.30	½	285	53	4.31	22.2	12.9	Weak curd, would not cook out
Propiolactone	8.85			0.091	22	<1035	4.33	½	390	55	4.33	19.4	20.0	Weak curd, would not cook out
Lactone	8.90			0.118	20	<960	4.68	¼	210	52	4.60	16.8	19.5	Normal curd
Lactone	9.06	0.02		0.118	20	<1020	4.80	¼	210	54	4.65	16.1	21.2	Normal curd
Lactone	9.06	0.02	3	0.120	20	<880	4.75	¼	210	56	4.71	16.2	21.8	Normal curd
Lactone	9.23	0.02		0.120	40	215	4.60	¼	65	54	4.70	13.5	26.0	Stringy, matted badly
Lactone	9.25	0.02		0.120	40	190	4.93	¼	125	55	4.67	16.3	21.2	Stringy, matted badly
Lactone	8.97			0.121	40	165	4.85	¼	75	50	4.70	16.0	22.3	Stringy, matted badly
Lactone	7.78			0.122	40	148	4.93	½	62	50	4.75	12.0	24.0	Stringy, matted badly
Lactone	8.86	0.02		0.1215	35	230	5.00	½	120	55	4.72	16.5	20.8	Normal curd
Lactone	8.95	0.02		0.122	36.5	200	5.00	½	160	54	4.72	17.2	20.6	Normal curd
Lactone	9.17		5	0.123	40	60	5.25	½	55	....	4.80	9.2	34.4	Stringy

[a] This factor multiplied by weight of solids in the milk equals weight of acidogen added.

[b] These experiments were run with low heat powder; in all others fluid skimmilk was used.

[c] Time from cutting curd to draining whey.

TABLE 2

Pilot plant production of Cottage Cheese with glucono-delta-lactone

Type of milk	% Solids of milk	% $CaCl_2$	Rennet (*ml. 1:10*)	Amount acidogen[a]	Coagulation temp. (*° F.*)	Coagulation time (*min.*)	Cutting pH	Knife size (*in.*)	Cooking time[b] (*min.*)	Cooking temp. (*° F.*)	Final pH	Curd Yield (*%*)	Curd Solids (*%*)	Remarks
Fluid	9.15	0.02		0.125	70	<1008	4.55	½	210	140	4.48	14.1	24.5	Normal curd
Fluid	9.17			0.123	94	255	4.73	½	120	128	4.50	16.2	22.0	Normal curd
Fluid	8.62			0.123	72	927	4.52	¼	186	120	4.52	18.5	22.8	Normal curd
Powder	9.00	0.02		0.123	72	960	4.60	¼	190	115	4.60	18.2	22.6	Cudl not uniform
Powder	9.00			0.123	72	997	4.65	¼	215	122	4.68	17.8	23.1	Curd not uniform
Powder	9.20	0.02		0.125	94	370	4.75	½	177	140	4.59	13.0	25.2	Curd not uniform
Powder	9.78		2	0.125	97	160	4.93	½	133	120	4.55	14.0	23.3	Normal curd

[a] This factor multiplied by weight of solids in the milk equals weight of acidogen added.
[b] Time from cutting curd to draining whey.

equipment, and culture will entirely offset the added cost of the acidogen. However, the convenience and reliability of this method may appeal to some. The acidogen process obviously will not be affected by antibiotics, bacteriophage, and the other vicissitudes to which bacteria are subject. The acidogen process makes it possible to produce Cottage Cheese in remote areas where cultures and skilled cheese makers are not easily obtained, and a long processing time is inconvenient. Also, the acidogen process is more easily controlled and duplicated than the lactic starter process. This should make it more readily adaptable to mechanized cheese making. It is possible that this process will also provide a way to study the effect of selected strains of bacteria on the development of body, texture, and flavor in various types of cheese.

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REFERENCES

(1) Brodie, A. F., and Lipmann, F. Identification of a Gluconolactonase. J. Biol. Chem., 212: 677. 1955.

(2) Carothers, W. H., Dorough, G. L., and VanNatta, F. J. Studies on Polymerization and Ring Formation. X. The Reversible Polymerization of Six-Membered Cyclic Esters. J. Am. Chem. Soc., 54: 761. 1932.

(3) Hucker, G. J., and Marquardt, J. C. The Effect of Certain Lactic Acid Producing Streptococci upon the Flavor of Cheddar Cheese. New York Agr. Expt. Sta., Tech. Bull., 117: 1. 1926.

(4) Johansson, H., and Sabelius, H. Uber die Verseifung von Glykilid und Lactid in Saure Losung. Ber., 52: 745. 1919.

(5) Jungfleisch, E., and Godchot, M. Sur le Dilactide droit. Comp. rend., 141: 111. 1905.

(6) Jungfleisch, E., and Godchot, M. Sur le Dilactide de l'Acide Lactique gauche. Comp. rend., 142: 637. 1906.

(7) Mabbitt, L. A., Chapman, H. R., and Berridge, N. J. Experiments in Cheese-Making Without Starter. J. Dairy Research, 22: 365. 1955.

(8) Mojonnier, Timothy, and Troy, H. C. The Technical Control of Dairy Products. Mojonnier Bros. Co., Chicago, Illinois. 1922.

(9) Percival, E. G. V. Structural Carbohydrate Chemistry. p. 33. Prentice-Hall, New York. 1950.

(10) Sammis, J. L. The Chemistry of Milk Curdling. p. 171. 24th Ann. Rept., Agr. Expt. Sta., Univ. of Wisconsin. 1907.

(11) Schneider, A. K. Polymers of High Melting Lactide. U. S. Patent 2,703,316. 1955.

(12) Tretsven, Wayne I. Evaluation of Procedure for the Manufacture of Cottage Cheese. Milk Plant Monthly, 36(11): 28. 1947.

(13) Van Slyke, L. L., and Publow, C. A. The Science and Practice of Cheese Making. p. 402. Orange Judd Company, New York. 1909.

(14) Walker, W. O. A Rapid Method for Determining the Percentage of Casein in Milk. Ind. Eng. Chem., 6: 131. 1914.

EFFECT OF CHANGING THE FAT AND NONFAT SOLIDS OF MILK[1]

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SUMMARY

Milk containing 2 and 3% fat was more acceptable in flavor than milk containing 4% fat, when each was standardized to 12, 13, 14, and 15% total solids. No statistically significant differences in flavor were observed for milk containing 2 or 3% fat together with 11 or 12% nonfat milk solids, but these were more acceptable in flavor than regular unfortified University plant milk containing 3.7% fat and 9.2% nonfat milk solids. By comparison, the regular plant milk tasted watery and flat.

The total amount of fat and nonfat solids (NFS), as well as the ratio of fat to NFS, is quite variable in market milk.

In 1958, when this study was initiated, there was a very large surplus of nonfat milk solids. The study to determine the acceptability of milk and/or milk beverages in which both the fat and NFS were standardized to definite levels had the following objectives: (a) To find a method for placing into food channels the surplus NFS, (b) to find a method of placing before the public a unit volume of milk of definite nutritional value.

Industry has had fine success in marketing nonfat milk when NFS are increased to approximately 10%. Another milk beverage, "2-10" (2% fat and 10% NFS), is popular with the housewife. The increase in the NFS eliminates to a considerable degree the flatness and watery flavor inherent in nonfat milk.

After this study was under way, Stull and Hillman (4) reported, "Many people can differentiate between milk beverages with variations of 0.5 and 1.0% in fat and NFS, respectively." They found, by paired preference test, that beverages with 0.5 and 1.0% added NFS were preferred. However, they found no significant difference in preference for a regular whole milk (3.5% fat and 8.5% NFS) when compared with a low-fat beverage (2.0% fat and 10% NFS).

EXPERIMENTAL PROCEDURE

Source and treatment of milk. The milk used in the experiment was a mixture of evening and morning milk from the University herd of Holstein, Guernsey, and Jersey cattle. The milk was standardized before pasteurizing to 3.7% fat and the NFS content ranged from 9.2 to 9.3%.

The milk was vat-pasteurized at 143-145° F. for 30 min. as a part of the regular University dairy plant operation. After the milk was cooled in the vat

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to 120-125° F., 10 gal. were drawn for each trial and immediately separated. The cream contained 13 to 20% fat. Immediately after the separation was completed the cream was homogenized at 1,800-2,000 p.s.i. by use of a Multi-Flo homogenizer. The homogenized cream and skimmilk were cooled in flowing water to approximately 55° F. and then placed in a 35-40° F. refrigerator until the next day. A portion of the skimmilk was either standardized to about 20% NFS by the addition of instantized NFS or it was condensed to approximately 25% total solids (TS) under 24-24.5 in. of vacuum in a 16-in. vacuum pan.

Levels of standardization. The milk for each trial was standardized to yield samples of the following composition:

Group 1 12% TS		Group 2 13% TS		Group 3 14% TS		Group 4 15% TS	
% Fat	% NFS	% Fat	% NFS	% Fat	% NFS	% Fat	% NFS
2	10	2	11	2	12	2	13
3	9	3	10	3	11	3	12
4	8	4	9	4	10	4	11

The standardization was accomplished by suitable combinations of cream, normal skimmilk, condensed or reinforced skimmilk, and/or distilled water.

Analysis for fat and total solids. The products used in preparing milks were tested for fat in duplicate by a modified Babcock fat test (2) and for TS (1) in duplicate by drying 2-g. samples at 100° C. for 5 hr. The standardized milks contained the desired fat content and total solids content within ±0.2%.

Judging panels. Six members of the departmental staff experienced in the judging of milk ranked the samples as to preference. The samples in Trials 3, 4, 5, and 6 were also ranked as to preference by 38 individuals who had had no previous experience in the judging of milk. Not all individuals were available to judge all replications. The six-member panel will be referred to as the T.P. (technical panel) and the other panel members as C.P. (consumer panel).

TABLE 1

Summation of ranks of samples within groups

Group	1 (12% TS)			2 (13% TS)			3 (14% TS)			4 (15% TS)		
Sample No.[a]	2-10	3-9	4-8	2-11	3-10	4-9	2-12	3-11	4-10	2-13	3-12	4-11
	Trial No. 1 (replicated 5 times)[b]											
Sum of ranks	40.5	45.0	76.5	41.5	43.5	71.0	46.5	49.0	60.5	61.0	40.5	60.5
W values[c]		0.5277			0.4016			0.0832			0.1876	
Level of significance		P<0.01			P<0.01			N.S.			P<0.01	
	Trial No. 2 (replicated 6 times)[b]											
Sum of ranks	43.5	64.0	96.5	60.0	59.0	85.0	67.0	51.0	74.0	68.0	55.0	69.0
W values[c]		0.6178			0.1877			0.1357			0.0596	
Level of significance		P<0.01			P<0.01			P<0.01			N.S.	
Total sum of ranks	84.0	109.0	173.0	101.5	102.5	156.0	113.5	100.0	134.5	129.0	95.5	129.5

[a] The first number indicates per cent fat; the second number indicates per cent NFS.

[b] Trial No. 1—additional NFS supplied from skimmilk reinforced with instantized NFS and Trial No. 2—from condensed skimmilk.

[c] Coefficient of concordance, *W* value, measures the degree of significance between the most acceptable and the least acceptable samples.

TABLE 2

Summation of ranks of samples selected from four groups with 12, 13, 14, and 15% total solids and 2, 3, and 4% fat samples in each group

Sample numbers ...	2-10	3-10	2-11	3-11	2-12	3-12	3.7-9.2[a]	*W* values[b]	Level of significance
Trial No. 3 (replicated 8 times)[c]									
T.P.[c] sum of ranks	214.0	193.0	144.5	141.0	144.5	129.0	.....	0.158	$P<0.01$
C.P.[d] sum of ranks	229.5	236.5	205.5	186.0	192.5	207.0	.....	0.031	N.S. at 5% level
Total sum of ranks	443.5	429.5	350.0	327.0	337.0	336.0	.....	0.068	$P<0.01$
Trial No. 4 (replicated 6 times)[e]									
T.P. sum of ranks	153.5	129.5	94.0	112.5	130.0	125.0	207.5	0.2440	$P<0.01$
C.P. sum of ranks	246.5	198.5	184.5	169.0	176.0	158.5	295.0	0.2001	$P<0.01$
Total sum of ranks	400.0	328.0	278.5	281.5	306.0	283.5	502.5	0.2044	$P<0.01$
Grand sum of ranks	843.5	757.5	628.5	608.5	643.0	619.5			

[a] Homogenized milk from the University dairy plant.
[b] Coefficient of concordanse, *W* value, measures the degree of significance between the most acceptable and least acceptable samples.
[c] Technical panel.
[d] Consumer panel.
[e] Trial 3—additional NFS supplied from skimmilk, reinforced with instantized NFS and Trial 4—from condensed skimmilk.

Judging procedure and analysis. A disinterested member of the staff coded and randomized the samples for each period of judging. In Trials 1, 3, and 5 the added NFS required to standardize the samples to the desired levels were supplied from skimmilk reinforced with instantized NFS. In Trials 2, 4, and 6 the additional NFS were supplied with condensed skimmilk.

Following the standardization for any given replicate of a trial, the samples were coded and randomized. In Trials 1 and 2 (Table 1) the groups were also randomized. Two groups (Table 1) were judged on the day of preparation and the remaining two groups the next forenoon. In Trials 3, 4, 5, and 6 the entire replicate was judged at one time.

The samples were ranked as to preference. The best-liked sample in a group (Table 1) or sample in a replicate (Tables 2 and 3) was given a rank of 1, the second best a rank of 2, etc.

W values found by applying the coefficient of concordance (3) were converted to *Z* values to determine the level of significance between the most acceptable and the least acceptable sample.

RESULTS AND DISCUSSION

The study of the samples presented in Table 1 was used to determine if differences existed in preference for milks in which the fat:NFS ratio varied within a TS grouping. The milks containing 2 and 3% butterfat were more acceptable than those containing 4% fat at all levels of TS studied. Samples 2-10, 2-11, 3-10, 2-12, 3-11, and 3-12, where the first number represents per cent fat and the second NFS (Table 1), were preferred within their individual total solids group. No differences in preferences were noted between Samples 2-11 and 3-10 or between 2-12 and 3-11.

TABLE 3
Summation of ranks for milk samples of composition selected as most accepetable in Trials 3 and 4

Sample No.	3-10	2-11	3-11	2-12	3-12	3.7-9.2[a]	Coefficient of concordance W values	Level of significance
			Trial No. 5 (replicated 3 times)[e]					
T.P.[c] sum of ranks	.....	38.0	34.0	38.0	30.0	70.0	0.5224	$P<0.01$
C.P.[d] sum of ranks	.....	45.5	57.5	55.0	53.0	74.0	0.1233	$P<0.01$
Total sum of ranks	.....	83.5	91.5	93.0	83.0	144.0	0.2399	$P<0.01$
			Trial No. 6 (replicated 6 times)[e]					
T.P. sum of ranks	146.5	112.5	111.0	110.0	105.5	149.5	0.0925	$P<0.01$
C.P. sum of ranks	139.0	116.5	105.5	89.5	89.5	132.0	0.1229	$P<0.01$
Total sum of ranks	285.5	229.0	216.5	199.5	195.0	281.5	0.1012	$P<0.01$
Grand sum of ranks		312.5	308.0	292.5	278.0	425.5		

[a] Homogenized milk from the University dairy plant.
[b] Coefficient of concordance, W value, measures the degree of significance between the most acceptable and the least acceptable samples.
[c] Technical panel.
[d] Consumer panel.
[e] Trial No. 5—additional nonfat solids supplied from skimmilk reinforced with instantized nonfat milk solids and Trial No. 6—from condensed skimmilk.

The composition of milks judged as most acceptable within given total solids groups (Table 1) were compared for acceptability. The effect of total solids is clearly evident in Trials 3 and 4 (Table 2). Samples 2-10 and 3-10 were the least acceptable. Both were considered flat or watery by comparison with the other samples. The 2-10 sample probably rated low because of the low TS, and 3-10 because it tasted less sweet.

Milks of a composition of 2-11, 3-11, 2-12, and 3-12 were compared, together with the regular University homogenized pasteurized milk (3.7% fat, 9.2-9.3% NFS) (Table 3). Samples 2-11, 3-11, 2-12, and 3-12 were difficult to distinguish flavorwise. The regular milk from the University plant was consistently criticized as being thin or watery, although it is not so criticized when not being compared with the standardized milk of higher TS. Though the University milk is considered a very good milk, apparently it could be improved by reducing the fat and increasing the NFS content.

Stull and Hillman (4) found no difference in flavor preference when 2-10 milk was compared with regular whole milk (3.5% fat, 8.5% NFS). However, their milks were standardized to only 12% TS; whereas, the 2-10 samples in our experiment were not compared with the regular standardized University milk which contained 3.7% fat and 9.2-9.3% NFS.

At the TS ranges of 13, 14, and 15% the panel members did not readily distinguish differences in flavor between the most acceptable milks, 2-11, 3-11, 2-12, and 3-12 (Table 3). These results vary somewhat from the results reported by Stull and Hillman (4), that many people can differentiate between milk beverages with variations of 0.5 and 1.0% fat and NFS, respectively. This does not appear to be so easy at TS levels of 13% or higher.

The results indicate that reducing the fat to 2 and/or 3% and raising the TS content to 13, 14, or even 15% may increase consumer acceptance (Table 2 and 3). Economically, increasing the NFS to definite and higher levels would eliminate any NFS surpluses. Such standardization appears to improve the flavor and certainly would improve and make more uniform the nutritional quality of a unit volume of milk.

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REFERENCES

(1) ASHWORTH, U. S. A Method for Selecting Drying Conditions for the Determination of the Total Solids in Milk. Proc. 38th Ann. Meeting, Western Div., American Dairy Sci. Assoc., Pullman, Washington. July 15-17, 1957.

(2) HERREID, E. O. Testing Milk and Milk Products for Fat and Total Solids. University of Ill., Coll. of Agr., Circ. 630. 1948.

(3) KENDALL, N. G. Rank Correlation Methods. Charles Griffin and Co., Ltd., London, p. 80-89. 1948.

(4) STULL, J. W., AND HILLMAN, J. S. The Consumer Acceptance of Milk Beverages as Affected by Fat and Solids-not-fat Content. J. Dairy Sci., 42: 1743. 1959; 43: 945. 1960.

PREDOMINANT BACTERIA IN THE RUMEN OF CATTLE ON BLOAT-PROVOKING LADINO CLOVER PASTURE [1]

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SUMMARY

A study was made on the microorganisms in the rumen of six yearling steers pastured on bloat-provoking Ladino clover. Three animals were selected for study on the basis of being good bloaters and three were nonbloaters. Studies on the bloaters were made before and 24 hr. after they were treated orally with 50 mg. of procaine penicillin to prevent bloat. There were no significant differences between animal groups in pH of ruminal contents, counts of cellulolytic bacteria, total anaerobic bacterial counts, or the occurrence of 11 presumptively identified bacterial groups. Counts of facultatively anaerobic streptococci were significantly depressed in the penicillin-treated group, but no other effects of treatment appeared to be significant. The means of the total anaerobic bacterial counts of the bloaters were higher than those of the two other groups, but the difference only approached significance. It is evident that the occurrence of bloat in animals consuming Ladino clover is not related to large differences in the numbers or species of ruminal microorganisms.

The bacterial flora of the rumen of these Ladino clover–pastured steers, culturable in rumen fluid agar medium, differed somewhat from usual, in that the total anaerobic counts were high (5.8-8.0 billion per gram), the proportions of cellulolytic bacteria (ruminococci and *Bacteroides succinogenes*) were very low, a species of anaerobic homofermentative lactobacilli was among the predominant bacteria, and the proportions of the genus *Butyrivibrio* were high and those of the genus *Lachnospira* were unusually high.

Numerous studies have suggested that ruminal bacteria are intimately involved in the etiology of bloat. Barrentine, Shawver, and Williams (1) showed that certain antibiotics were effective over a short period in the control of bloat caused by legumes. Hungate *et al.* (15) showed that the rate of both acid and gas production are greater in rumen contents from bloating animals than from nonbloating animals fed the same Ladino clover pasture. However, several workers have shown that the ruminant can eliminate free gas from the rumen at a far faster rate than necessary to prevent bloat, so that the rapid microbial production of gas alone is not considered a primary cause of bloat.

It now is generally agreed that entrapment of ruminal gases in a stable foam which can not be eliminated is the main cause of bloat [see Johns (17)]. However, little is known of the factors involved in the production of stable foam. Hungate *et al.* (15) suggested that bacterial slime production was a cause of the stable foam in bloat due to legumes. Jacobson *et al.* (16) found the degree of encapsulation of ruminal bacteria to be highly correlated with

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bloat of the feedlot type, and Gutierrez *et al.* (12) suggested that the increased numbers of slime-producing *Streptococcus bovis* and *Peptostreptococcus elsdenii* producing a filamentous mat in animals bloated on the feedlot type ration might be involved in the production of a stable foam. Gutierrez, Davis, and Lindahl (11) found considerable amounts of slime to be produced by strains similar to *Butyrivibrio fibrisolvens* and *Bacteroides ruminicola* during growth on alfalfa saponin, and suggested that this reaction could be of significance in bloat.

Mangan (18) indicated that cytoplasmic protein of the plant is of major importance as a foaming agent and showed that chloroplasts from red clover have a high anti-foaming property. This appeared to be due to their high concentration of lipids, since chloroplasts washed with acetone and ether had no anti-foaming properties. Mangan, Johns, and Bailey (19) suggest that these chloroplasts play an important part in the etiology of bloat and suggest that ruminal bacteria normally modify the chloroplast lipid in some way to lessen its anti-foaming property. They suggest that penicillin may inhibit these bacteria and thereby control bloat. It can also be inferred from this hypothesis that ruminal bacteria in nonbloating animals fed bloat-provoking legumes differ in their metabolism of chloroplast lipid from bacteria in bloating animals on the same diet.

If the metabolic activities of ruminal bacteria are intimately involved in the etiology of bloat, then differences in rumen bacterial metabolism in bloating and nonbloating animals on the same diet might reflect a qualitative and/or quantitative difference in the species of bacteria active in the rumen.

The present study was undertaken to determine if differences could be found in the predominant bacterial groups present in bloating and nonbloating cattle fed on Ladino clover pasture. The effect of penicillin on the incidence of bloat and the ruminal population also was examined.

EXPERIMENTAL PROCEDURE

Yearling steers were pastured on Ladino clover at Mississippi State University for 90-min. periods beginning at 7:30 A.M. and 2:30 P.M. daily. Animals were selected for study on the basis of being prone to bloat or resistant to bloat. The determination of whether animals bloated was based on visual observations only. Table 1 shows the incidence of bloat and time of penicillin treatment and sample collection of ruminal contents.

Sample collection, methods of dilution, and inoculation of culture media, and the total anaerobic count medium (rumen fluid-glucose-cellobiose medium) were those previously described by Bryant and Burkey (3), except that rumen fluid from Ladino clover–fed cattle was used in the medium. Other media inoculated were cellulose liquid medium (3), aerobic medium identical with the anaerobic medium except that Na_2CO_3, CO_2, and cysteine-HCl were deleted, and the selective lactobacillus medium of Rogosa *et al.* (21).

The pH of the whole rumen contents was determined with a glass electrode pH meter within 15 min. of collection.

TABLE 1

Occurrence of bloat and time of penicillin dosing and sample collection in selected bloating and nonbloating steers

Date	Bloating steers						Nonbloating steers					
	27		4		49		33		34		50	
	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.
June 4	+[a]	+	+	0	+	+	0	0	0	0	0	0
5	+	+	0	0	+	+	0[b]	0	0	0	0	0
6	+	+	+	0	+	0	0	0	0	0	0	0
7	+	+	+	+	0	+	0	0	0[b]	0	0	+
8	+	+	+	+	+	+	0	0	0	0	0	+
9	+[b]	+	+	+	+	+	0	0	0	0	0	+
10	+[c]	0	+	+	+	+	0	0	0	0	0	0
11	0[b]	0	+	+	+	+	0	0	0	+	0	0
12	+	+	+	+	+	+	0	0	0	0	0	0
13	+	+	+[b]	+	+	+	0	0	0	+	0	0
14	(out)		+[c]	+	+	+	+	0	0	+	0	0
15			+	+	+	+	+	0	(died)		0[b]	0
16			+	+	+	+	+	+			0	0
17			+	0	+[b]	+	+	+			0	0
18			+	+	+[c]	0	+	+			0	0
19			+	+	0[b]	0	+	0			0	+
20			+[c]	+	0	0	+	0			0	0
21			0[b]	0	0	0	+	0			0	0
22			0	0	+	0	+	0			0	0
23			0	0	0	0	0	0			0	0
24			+	+	+	0	+	0			0	0

[a] + = bloated, 0 = not bloated.

[b] Sample was collected on this date at 1 P.M.

[c] The animal was given orally and by capsule 50 mg. of procaine penicillin at 1 P.M. on this date.

Colony counts were obtained from duplicate plates of the lactobacillus medium, and aerobic medium inoculated with appropriate dilutions and incubated for 72 hr. at 37° C. The counts of cellulolytic bacteria were obtained by the most-probable-number method previously described (8). Anaerobic colony counts reported were means of counts from five roll tube cultures at each of two dilutions (either 10^{-8} and 3×10^{-9} or 3×10^{-9} and 10^{-9}).

Representative colonies from the aerobic plates were studied by microscopic observations of gram stains. Fifty-five colonies were picked from the anaerobic medium inoculated with each sample and studied for colony type, morphology, motility, gram reaction, final pH, and appearance of growth in glucose liquid medium, relations to oxygen, H_2S production, gas production, and cellulose digestion, as previously indicated (6).

These characteristics were used to presumptively identify the strains, as indicated in Table 2. Reference to detailed descriptions of the characteristics of species to which Groups 1, 2, 3, 5, 6, 7, 8, and 9 were presumptively assigned are given by Bryant (2). Further characteristics of representative strains of Groups 4 and 10 were described (2).

Representative strains of Groups 3, 5, 8, and 11 have been studied in detail in all characteristics utilized by Bryant *et al.* (7), except fermentation products and Groups 3, 5, and 8 corresponded to the presumptive identification given in Table 2 (unpublished data). Strains of Group 11 varied greatly in charac-

TABLE 2

Some characteristics used in the presumptive identification of bacterial groups[a]

Group	Presumptive identification	Morphology	Gram stain	Motility	H_2S	Cellulose	Gas	Final pH
1	*Ruminococcus* sp.	Cocci	±[b]	−	−	+	∓	5.0-7.0
2	*Bacteroides succinogenes*	Pleomorphic rods	−	−	−	+	−	5.0-6.0
3	*Bacteroides ruminicola*	Pleomorphic rods	−	−	±	−	−	5.0-6.0
4	*Lactobacillus* sp.	Medium-sized rods	+	−	−	−	−	4.3-4.5
5	*Selenomonas*	Crescentic rods	−	+	+	−	−	4.3-4.5
6	*Succinivibrio*	Small, helicoidal, pointed end rods	−	+	−	−	−	5.0-6.0
7	*Butyrivibrio*	Medium or small-sized curved rods	−	∓	∓	∓	+	5.0-6.0
8	*Butyrivibrio* (atypical)	Medium or small-sized curved rods	−	+	∓	−	±	4.8-6.5
9	*Lachnospira*[c]	Medium-sized curved rods	±	+	−	−	+	4.8-5.2
10	Similar to Strain B385	Large curved rods	−	+	−	−	+	4.7-5.1
11	Unidentified group	Fair- to large-sized spindle- to lancet-shaped rods	−	+	±	−	±	5.0-6.0

[a] All strains isolated were strict anaerobes.

[b] The ± under gram stain refers to variability within a strain and between strains. In other cases ± refers to variability between strains, but most strains are positive. ∓ means that most strains are negative.

[c] The filamentous, woolly colony is very typical in this group.

teristics, including H_2S, amount of gas, final pH, ammonia production from trypticase, sugars fermented, and colony size, and although flagella were very difficult to stain, they appeared to be mainly monotrichous and polar, or polar tufts, and a few strains had peritrichous flagella. Further studies on the identity of this group are warranted, as similar strains have been found in small numbers in the rumen of cattle on many dietary regimes.

RESULTS

The data in Table 1 indicate that the steers selected as bloaters bloated after almost every feeding period except when penicillin was given. The first capsule of penicillin given to Steer 4 did not prevent bloat. It is possible that the capsule was not swallowed. Otherwise, the penicillin appeared to prevent bloat from three to seven feeding periods.

The data show that steers selected as nonbloaters did bloat to some extent, but did not bloat at least for several days before and after sampling. Bloat was the apparent cause of death of Animal 34.

Cursory direct microscopic observations on protozoa revealed no differences between animal groups. All animals contained large holotrichs and both small and large oligotrichs. *Entodinium* was by far the most numerous genus.

Application of the Duncan Multiple Range Test to data shown in Table 3

TABLE 3

Bacterial culture counts and pH values of rumen content samples from bloating, nonbloating, and penicillin-treated steers pastured on Ladino clover

Animal No.	pH	Cellulolytic bacteria	Aerobic count	Anaerobic count
		(millions per gram)	*(millions per gram)*	*(billions per gram)*
		Nonbloaters		
33	5.8	95	0.22	7.3
34	6.5	49	13.00	4.7
50	6.0	33	14.00	5.3
Mean	6.1	59	9.1	5.8
		Bloaters		
27	6.2	79	14.0	8.0
4	6.4	130	18.0	8.8
49	6.3	920	18.0	7.2
Mean	6.3	376	16.7	8.0
		Penicillin-treated bloaters		
27	6.4	92	3.0	2.9
4	6.0	>180	4.5	8.3
49	5.9	>180	1.6	6.1
Mean	6.1	>151	3.0	5.8

did not reveal any significant differences in pH values, counts of cellulolytic bacteria or anaerobic counts of bacteria for the samples of rumen contents collected from nonbloating animals, bloating animals, or the latter animals treated with penicillin. The mean of the anaerobic counts from the bloaters was higher than those of the other groups and the difference approached significance at the 5% level.

The mean of the aerobic counts was significantly lower (5% level) in the penicillin-treated animals. Observations on colony type and gram stains of representative colonies indicated that short-chain streptococci predominated (60-95% of colonies) in all plates of aerobic medium. Therefore, this effect of penicillin was mainly on streptococci.

All counts of lactobacilli able to grow under aerobic conditions were less than 1,000 per gram.

There were no significant differences in the occurrence of the bacterial groups isolated from the different animal groups (Table 4).

TABLE 4

Groups of bacteria isolated from three nonbloating and three bloating steers and the latter three steers treated with penicillin to prevent bloat
(All animals on Ladino clover pasture)

Animal group	Per cent of strains					
	Nonbloaters		Bloaters		Penicillin-treated	
Bacterial group	Mean	Sample range	Mean	Sample range	Mean	Sample range
1	1.4	0– 2.1	0	0	0.6	0– 1.8
2	0.7	0– 2.1	0	0	1.3	0– 2.1
3	15.1	2.2–36.2	5.9	3.8–10.2	13.5	3.6–25.5
4	8.2	0–14.9	7.2	2.0–10.2	7.1	3.8–12.8
5	3.4	1.9– 6.4	2.6	0– 6.0	1.3	0– 3.8
6	0	0	0.7	0– 2.0	0.6	0– 1.9
7	29.5	10.6–42.2	27.0	12.2–34.0	25.8	16.4–35.8
8	3.4	0– 5.6	13.2	6.0–24.5	15.5	7.5–29.1
9	17.8	15.6–19.1	25.0	22.4–28.0	22.6	14.9–30.9
10	11.0	0–27.8	4.6	2.0– 7.5	1.3	0– 4.3
11	6.2	1.9–15.6	6.6	3.8–10.0	7.7	4.3–12.7
12[a]	3.4	0– 6.4	7.2	5.7–10.2	2.6	0– 5.7
Total strains	146		152		155	

[a] Miscellaneous ungrouped strains.

Also, when attempts were made to correlate the number of strains having certain characteristics with bloaters and nonbloaters, with or without regard to presumptive species, no correlations were evident. Characters studied included the following: Gram reaction, motility, amount of H_2S produced, cellulose digestion, amount of gas produced, final pH in glucose medium, and visible slime in glucose medium.

DISCUSSION

As the so-called nonbloating steers actually did bloat to some extent, it is possible that, if more rigorous selection of nonbloaters had been possible, significant differences in the ruminal flora of the animals would have been evident. However, animals differ only in their relative susceptibility to bloat and most animals bloat at least occasionally.

The fact that no significant quantitative or qualitative differences were seen in the ruminal microorganisms cultured from bloating and nonbloating cattle indicates that gross changes in the numbers or species of microorganisms are not a prerequisite to bloat. However, it may be that some significant com-

ponent of the flora was not grown in the culture media utilized, or that a significant component of the flora does not produce enough viable units to be found among the predominant colonies from which strains were isolated.

It is also a possibility that strains within one or more of the bacterial groups identified differed in their metabolic characteristics in ways not detectable with the few characteristics studied, and that the presence or absence of this character would correlate with the animals' ability to bloat. It is common knowledge that there are wide strain variations within certain species of ruminal bacteria (2), and various factors in individual animals might cause the growth of strains within a bacterial species which differ in certain significant metabolic characteristics. It was shown that only two of 16 strains of *Butyrivibrio* selected for further study fermented a composite saponin from alfalfa (Mr. Ivan Lindahl kindly supplied the saponin). If all strains isolated could have been studied for this character, perhaps a difference in the ability to ferment this compound would have been apparent in the bacteria from the bloaters and nonbloaters.

The above discussion, together with the fact that no difference in the occurrence of bacterial species was evident, suggests that future attempts to correlate culturable ruminal bacterial groups with bloat should involve the use of culture media which selectively enumerate metabolic groups of bacteria, rather than the relatively nonselective culture methods, as used in the present study, which enumerated mainly taxonomic groups. For example, it would be of interest to compare numbers and species of saponin-fermenting, pectin-fermenting, protein-hydrolyzing, and fat-catabolizing bacteria in the rumen of bloating and nonbloating cattle on the same ration. All of these materials have been suggested as being related to stable foam production in the rumen—see, for example, Gutierrez *et al.* (11), Conrad *et al.* (9), and Mangan (18).

Since the difference in anaerobic bacterial counts between the bloaters and nonbloaters approached significance, it is possible that the higher count in the bloaters would have been significant if more samples had been studied. However, the study was designed especially to look for qualitative differences in the flora, and the time available in Mississippi to the senior author was not sufficient for a longer study. The greater magnitude of the ruminal fermentation in bloating than in nonbloating animals, with no qualitative difference in fermentation products reported by Hungate *et al.* (15), might have been due to factors resulting in the growth of larger numbers of bacteria in the rumen of bloaters.

It was rather surprising that, although the one dose of penicillin prevented bloat in each of three animals, there was little apparent effect on the numbers or species of ruminal bacteria cultured. The numbers of facultatively anaerobic streptococci were significantly depressed. This agrees with results of Hartman *et al.* (13). However, the numbers of streptococci were quite low as compared to anaerobic bacteria (approximately a 500-fold difference), and it seems extremely unlikely that they contributed to bloat under the present conditions. The mean of counts of total anaerobes in the penicillin-treated animals was

lower than in the same animals not treated with penicillin. However, this difference only approached the 5% level of significance. This may be the reason for the effect of penicillin treatment.

These results with penicillin treatment suggest that the effectiveness of penicillin in controlling bloat could not be explained on the basis of large differences in numbers or species of rumen bacteria. This observation is consistent with the comparison of the flora and fauna of bloating and nonbloating steers, in which there were little or no differences. It may be that the metabolism of the flora was altered, as shown in the study of Mangan, Johns, and Bailey (19), but that a concurrent alteration in species present is not necessary. It is also possible that because of the large numbers of microbes present, the 24-hr. period between penicillin treatment and rumen sampling was not a long enough period to allow for death or diluting out of species inhibited by penicillin.

The present results on penicillin seem to be in essential agreement with those of Horn, Snapp, and Gall (14), who found that penicillin up to 200 mg. per day had little effect on the anaerobic flora of yearling steers. However, these workers fed the antibiotic for 2 wk. before sampling, which would allow the ruminal flora time for adaptation to the antibiotic—see, for example, Mangan, Johns, and Bailey (19). Unpublished data of Bryant, Robinson, and Lindahl indicate that 75 and 150 mg. of procaine penicillin fed daily to two full-grown steers on a feedlot bloat-provoking ration had a drastic effect on the second day, in that total anaerobic counts and cellulolytic counts were strongly depressed and predominant species of bacteria were radically changed. After continued penicillin dosing of up to 200 mg. per day after the animal resumed eating, animals did not go off feed and the numbers and species of bacteria approached normal. This indicates that penicillin does have a drastic effect on the flora on first exposure if fed at a high enough level, but that the flora quickly adapts to the treatment. It is possible that a higher level of antibiotic is required to show a drastic effect on the flora of Ladino clover–fed animals than in animals on the feedlot bloat ration.

In comparing the cultural data collected from these Ladino clover–fed animals with data from animals on other rations, it is evident that the present animals had a higher total anaerobic count than animals on the usual ration containing large quantities of dry roughage and/or silage (4, 5). However, young calves (6, 8) and mature animals receiving a high ratio of concentrate to hay (4) and mature animals on lush bluegrass pasture (unpublished data of Bryant) can also have total counts as high or higher than the present animals. The results on total counts are in agreement with cultural results of Gall *et al.* (10) and direct microscopic counts of numerous workers (see, for example, 10, 20, 22), indicating that larger numbers of bacteria per unit volume or unit weight of ruminal contents are present when animals are on lush pasture than when fed dry roughage.

The proportions of certain bacterial species cultured from the present animals (Table 4) were somewhat different from those found in animals on a

wide variety of rations, previously studied in the senior author's laboratory. The following points should be noted: (1) The proportion of cellulolytic bacteria (ruminococci and *Bacteroides succinogenes*) was very low, even when compared with an all-concentrate ration (4). (2) The anaerobic homofermentative *Lactobacillus* sp. (Group 4) has been found only in animals on fresh-cut alfalfa (2) and in the present study. Perhaps their increased numbers are a response to the large amount of soluble carbohydrate one would expect in such rations. (3) The proportion of strains of the genus *Butyrivibrio* was quite high. (4) The proportions of the genus *Lachnospira* were much higher than have been obtained under any other conditions.

Under most dietary regimes studied, the most obvious and prevalent strains producing visible slime in glucose medium belong to the *Bacteroides ruminicola* group. In the Ladino clover–fed steers, strains of *Butyrivibrio* were more obvious in this respect.

Whether any of these peculiarities of the flora contribute to bloat is pure speculation, since the nonbloating animals also had them. It does seem probable that a very active flora suggested by the high cultural count is of significance and it is possible that the high percentage of gas-producing strains, in particular the genera *Butyrivibrio* and *Lachnospira*, is of significance.

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REFERENCES

(1) Barrentine, B. F., Shawver, C. B., and Williams, L. W. Antibiotics for the Prevention of Bloat in Cattle Grazing Ladino Clover. J. Animal Sci., 15: 440. 1956.

(2) Bryant, M. P. Bacterial Species of the Rumen. Bacteriol. Revs., 23: 125. 1959.

(3) Bryant, M. P., and Burkey, L. A. Cultural Methods and Some Characteristics of Some of the More Numerous Groups of Bacteria in the Bovine Rumen. J. Dairy Sci., 36: 205. 1953.

(4) Bryant, M. P., and Burkey, L. A. Numbers and Some Predominant Groups of Bacteria in the Rumen of Cows Fed Different Rations. J. Dairy Sci., 36: 218. 1953.

(5) Bryant, M. P., and Burkey, L. A. The Bacterial Flora in the Rumen of Heifers Fed a Ration of Alfalfa Silage. USDA, BDI-Inf-151. 1953.

(6) Bryant, M. P., and Small, Nola. Observations on the Ruminal Microorganisms of Isolated and Inoculated Calves. J. Dairy Sci., 43: 654. 1960.

(7) Bryant, M. P., Small, Nola, Bouma, Cecelia, and Chu, Hilda. *Bacteroides ruminicola*, sp. nov. and *Succinimonas amylolytica*, gen. nov.—Species of Succinic Acid–Producing Anaerobic Bacteria of the Bovine Rumen. J. Bacteriol., 76: 15. 1958.

(8) Bryant, M. P., Small, Nola, Bouma, Cecelia, and Robinson, I. M. Studies on the Composition of the Ruminal Flora and Fauna of Young Calves. J. Dairy Sci., 41: 1747. 1958.

(9) Conrad, H. R., Pounden, W. D., Bentley, O. G., and Fetter, A. W. Production of Gas in Rumen Fermentations and Stable Mass Formation from Alfalfa Fiber and Pectic Substances. J. Dairy Sci., 41: 1586. 1958.

(10) Gall, L. S., Burroughs, W., Gerlough, P., and Edgington, B. H. Rumen Bacteria in Cattle and Sheep on Practical Farm Rations. J. Animal Sci., 8: 441. 1949.

(11) Gutierrez, J., Davis, R. E., and Lindahl, I. L. Characteristics of Saponin-Utilizing Bacteria from the Rumen of Cattle. Appl. Microbiol., 7: 304. 1959.

(12) GUTIERREZ, J., DAVIS, R. E., LINDAHL, I. L., AND WARWICK, E. J. Bacterial Changes in the Rumen During the Onset of Feed-lot Bloat of Cattle. Appl. Microbiol., 7: 16. 1959.

(13) HARTMAN, P. A., JOHNSON, R. H., BROWN, L. R., JACOBSON, N. L., AND ALLEN, R. S. Effect of Diet and Feed Additives on Facultative Anaerobes in the Rumen. Report of Fifth Conf. Rumen Function. Ed. by N. R. Ellis, A.H.R.D., Beltsville, Md. 1960.

(14) HORN, L. H., SNAPP, R. R., AND GALL, L. S. The Effect of Antibiotics upon the Digestion of Feed Nutrients by Yearling Steers with Bacteriological Data. J. Animal Sci., 14: 243. 1955.

(15) HUNGATE, R. E., FLETCHER, D. W., DOUGHERTY, R. W., AND BARRENTINE, B. F. Microbial Activity in the Bovine Rumen, Its Measurement and Relation to Bloat. Appl. Microbiol., 3: 161. 1955.

(16) JACOBSON, D. R., LINDAHL, I. L., MCNEILL, J. J., SHAW, J. C., DOETSCH, R. N., AND DAVIS, R. E. Feedlot Bloat Studies. II. Physical Factors Involved in the Etiology of Frothy Bloat. J. Animal Sci., 16: 515. 1957.

(17) JOHNS, A. T. Recent Developments in Bloat Research. Vet. Revs. Annotations, 4: 17. 1958.

(18) MANGAN, J. L. Bloat in Cattle. XI. The Foaming Properties of Proteins, Saponins and Rumen Liquor. New Zealand J. Agr. Research, 2: 47. 1959.

(19) MANGAN, J. L., JOHNS, A. T., AND BAILEY, R. W. Bloat in Cattle. XIII. The Effect of Orally Administered Penicillin on the Fermentation and Foaming Properties of Rumen Contents. New Zealand J. Agr. Sci., 2: 342. 1959.

(20) MOIR, R. J. The Seasonal Variation in the Ruminal Microorganisms of Grazing Sheep. Australian J. Agr. Research, 2: 322. 1950.

(21) ROGOSA, M., MITCHELL, J. A., AND WISEMAN, R. F. A Selective Medium for the Isolation and Enumeration of Oral Lactobacilli. J. Dental Research, 30: 682. 1951.

(22) WILLIAMS, V. J., AND CHRISTIAN, K. R. Rumen Studies in Sheep. II. A Comparison Between Rumen Microbial End-Products in Thrifty and Ill-Thrifty Hoggets. New Zealand J. Sci. Technol., Sec. A, 38: 268. 1956.

A COMPARISON OF THE ALL-GLASS, SEMIPERMEABLE MEMBRANE, AND CONTINUOUS FLOW TYPES OF APPARATUS FOR IN VITRO RUMEN FERMENTATIONS [1, 2]

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SUMMARY

A comparison was made of three different types of apparatus commonly used for in vitro rumen fermentation studies. The types compared were the all-glass, the semipermeable membrane, and the continuous flow. Purified wood cellulose and several different types of hay were used as substrates, with cellulose digestion, total volatile fatty acid production, and ammonia nitrogen production (for the hay samples only) being used as criteria of microbial activity. No major differences were found between the different types of apparatus; however, the all-glass apparatus appears to be advantageous because of its simplicity.

For in vitro rumen fermentations Marston (11) used a suspension of rumen microorganisms in mineral mixture in a large glass container, tightly fitted with a metal lid and a rubber gasket with inlets and outlets for gas, solutions, electrodes, and a stirrer. Bentley *et al.* (1) used a simplified all-glass apparatus for in vitro rumen fermentations, with a more elaborate mineral media to meet the requirements of cellulolytic microorganisms, somewhat similar to the methods reported previously by Gray *et al.* (6) and by Burroughs *et al.* (2).

A different apparatus, utilizing a semipermeable membrane, has been devised by Louw *et al.* (10) in an attempt to simulate more closely the in vivo conditions. Modifications of the same method have been used by other workers (8, 15). Louw *et al.* (10) obtained better cellulose digestion with the semipermeable type of apparatus when compared to an all-glass apparatus.

Recent experiments carried out in this laboratory (14) indicated that the microorganisms responsible for the breakdown of cellulose in vitro in an all-glass type of in vitro rumen fermentation apparatus are authentic and representative of the rumen cellulolytic bacteria.

Due to the variety of the types of apparatus employed for studying biochemical and microbiological activities of rumen microorganisms, and to the different degrees of complexity, it was thought that a comparison between the two major types, i.e., the all-glass and semipermeable, would be of interest for continued work in this field. Further, a third technique, utilizing a semipermeable membrane suspended in a continuous flow of mineral mixture, was introduced in the comparison.

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[2] This work was supported in part by funds allocated to the Station on the recommendation of the North Central Technical Committee on Ruminant Nutrition (NC-25).

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EXPERIMENTAL PROCEDURE

Procedure and apparatus. Samples of rumen contents were drawn from a steer or sheep through a permanent rumen fistula, and strained through four layers of cheese cloth. Forty milliliters of the strained rumen liquor were used to inoculate the flasks or the semipermeable tubes which were previously filled with mineral-nutrient basal media, as previously described from this laboratory (1, 5), plus the substrate, and gassed continuously with CO_2.

The semipermeable apparatus was similar to that of Warner (15). Two gram substrate plus 40 ml. rumen liquor were mixed inside the cellophane dialyzing tube and suspended in 80 ml. of the basal media.

The continuous flow apparatus (Figure 1) is a modification of the above-

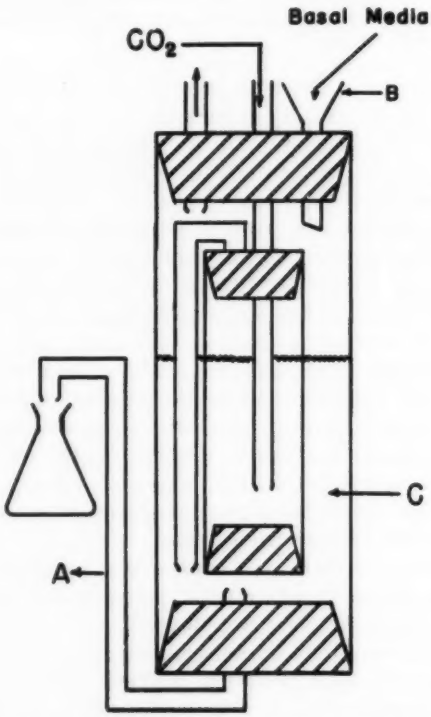

FIG. 1. Continuous flow type of in vitro rumen fermentation apparatus.
A. Siphon.
B. Inlet for basal media.
C. Basal media in outer jacket.

mentioned semipermeable apparatus. Siphon (A) was adjusted so that after suspension of the semipermeable bag and bubbling through CO_2, 80 ml. of basal media were present in the outer jacket (C). A small funnel, inserted

through inlet (B), delivered the CO_2 saturated mineral solution into the outer jacket (C), causing a continuous flow of this solution which was collected in an Erlenmeyer flask of suitable size.

The mineral solution was stored in a large 2-3 liter separatory funnel and the flow was roughly regulated by passing through a 26-gauge hypodermic needle. The pH of the solution collected in the Erlenmeyer flask was about 7.5, which was higher than the mineral media (6.9), due to standing and loss of CO_2. The mineral solution was made up in the same proportions as previously described (1) except for urea and *n*-valeric acid which were added at half the reported concentration, i.e., 63 mg. and 15 mg. of urea and *n*-valeric acid, respectively, per 100 ml. of solution. Samples were collected after 3, 8, 24, and 30 hr. The volumes of the collected samples were measured and aliquots were used for volatile fatty acids and ammonia-N determinations. After 30 hr. of fermentation the contents of the bags were quantitatively transferred into a bottle calibrated at 100 ml. and made to volume.

Cellulose determination. Digestion of Purified Cellulose[4] was measured by the volumetric method of Hershberger *et al.* (7). The Crampton and Maynard method (4) was employed for determining cellulose digestion of hays.

Total volatile fatty acids. Samples were deproteinized with 0.1 *N* HCl and steam distilled in a micro-Kjeldahl distillation apparatus (15), except for dialysates from the semipermeable systems, which were analyzed without prior deproteinization.

Ammonia nitrogen. Ammonia nitrogen was determined by the micro-diffusion method of Conway and O'Mally (3).

Direct microscopy. Microscopic examination of microorganisms from the different fermentation systems was undertaken at the end of each fermentation period. The slides were prepared by staining with the Gram-stain (Hucker modification).

RESULTS AND DISCUSSION

Table 1 gives the results of preliminary experiments using purified cellulose (Solka Floc)) as a substrate in the three types of apparatus. Cellulose digestion, as well as total volatile fatty acid production, were used as criteria of bacterial activity. Although there were variations in cellulose digestion from one experiment to another, which were mostly due to daily variations in the inoculum fermentation power, it can be seen that the three types of apparatus gave similar cellulose digestion, as indicated by both criteria.

Several varieties of hay, some at different stages of growth, were then chosen as substrates for a comparison of these three different types of apparatus. These results are presented in Table 2 and, as can be seen, there was little to prefer from one type of apparatus to another based on cellulose digestion, volatile fatty acids, or ammonia-N. The rates of volatile fatty acid production are compared (Figure 2) for fourth stage timothy hay, and the curves are typical of the other substrates. In the continuous flow apparatus the 3, 8,

[4] Solka Floc BW-40, Brown Company, Berlin, New Hampshire.

TABLE 1

Cellulose digestion and total volatile fatty acids (T.V.F.A.) produced in vitro from purified cellulose incubated with steer rumen fluid

Criteria	Expt. No.	Apparatus		
		All-glass	Cont. flow	Semipermeable
Cellulose digested *(g.)*	1	0.831	1.179	0.631
	2	1.313	1.140	.....
	3	1.386	0.858	1.153
	4	0.817	0.791	.....
	$\bar{x}$	1.087	0.992	0.892
T.V.F.A. (M.E.)	1	12.70	13.11	9.03
	2	15.21	13.87	10.13
	3	13.52	11.66	12.28
	4	11.05	11.14	.....
	$\bar{x}$	13.02	12.44	10.46

TABLE 2

Cellulose digestion, total volatile fatty acids, and ammonia-nitrogen produced by 30-hr. in vitro fermentations[a]

Substrate[b]	Apparatus	Cellulose digestion *(%)*	T.V.F.A.[d] (M.E.)[e]	Ammonia-N (M.E.)[e]
Mixed hay	All-glass	48.0 ± 1.2[c]	12.9	.....
	Continuous flow	48.6 ± 1.2	13.7	.....
Timothy hay First stage	All-glass	75.8 ± 4.0	12.0	5.3
	Continuous flow	73.7 ± 2.8	12.7	11.1
	Semipermeable	76.6 ± 0.3	12.1	4.0
Timothy hay Fourth stage	All-glass	37.4 ± 0.4	11.0	5.2
	Continuous flow	36.9 ± 0.4	10.3	12.8
	Semipermeable	38.8 ± 0.6	9.8	4.3
Alfalfa hay Second stage	All-glass	48.0 ± 1.5	11.2	5.5
	Continuous flow	51.6 ± 1.2	11.1	14.5
	Semipermeable	51.2 ± 0.3	10.4	5.2

[a] All results are the average of duplicate experiments.
[b] Two grams of each hay were added. Cellulose content of hay: Mixed hay, 25.0%; 1st stage timothy, 25.8%; 4th stage timothy, 29.7%; 2nd stage alfalfa, 25.1%.
[c] Standard deviation.
[d] Total volatile fatty acids—T.V.F.A.
[e] Milliequivalents—M.E.

and 24 hr. time samples represent only the volatile fatty acids in the collected fractions but not inside the bag, since no samples were obtained from the bags except at the end of the fermentation. The total volatile fatty acids reported in Tables 1 and 2 were obtained from inside and outside the bags at the end of 30 hr. of fermentation. Ammonia-N produced in the continuous flow apparatus was always higher than in the other systems. This is probably due to the higher over-all urea concentration to which the microorganisms were subjected (63 mg. urea per 100 ml. mineral solution).

Since it was thought that the rate of flow may affect cellulose digestion in the continuous flow apparatus, two experiments were carried out using varying rates of flow, so that the volume of solution collected varied from 400 to 1,600 ml. The results of these experiments suggested that the rates of flow, as indi-

cated by the volume collected, did not have any marked effect on cellulose digestion.

It is obvious that more work is needed to exploit the usefulness of the continuous flow apparatus. Using this apparatus, the pH need not be examined and adjusted during the fermentation period. However, as the present experiments suggest, it appears to have no advantages over the all-glass apparatus for fermentation periods of up to 30 hr. It may be more useful for in vitro experiments carried out for longer periods, 48-96 hr., particularly in view of the findings of Warner (15), that changes in the microflora were less evident in four-day incubations if the mineral media were changed several times during that period. However, the cellophane membrane can not be used for periods longer than 30 hr., since it is weakened considerably around the rubber stopper

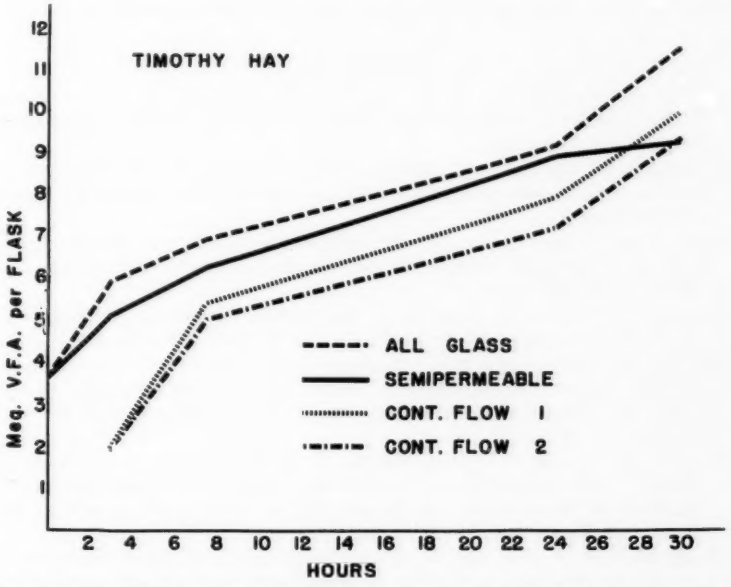

FIG. 2. Total volatile fatty acids produced in the three types of in vitro rumen fermentation apparatus.

at the lower end, probably due to bacterial attack. The use of other membranes should be attempted. A strip of the rumen wall epithelium may be useful in specific biochemical studies, since it may be involved in some biochemical reactions, as shown by Pennington (12, 13).

It should be borne in mind that with a continuously flowing mineral solution some of the essential micro-nutrients, required by the microorganisms and not found in the basal media, may be washed away. As a result, retardation of the fermentation may occur; however, this possible effect was not observed in the present experiments.

More efficient removal of end products that may be inhibitory to microbial activity is accomplished by this system, but the fact that it did not give better cellulose digestion than the all-glass apparatus suggests that end products such as volatile fatty acids are not inhibitory for cellulose digestion. This is in agreement with previous work from this laboratory (9), where a considerably higher concentration of volatile fatty acids did not inhibit the cellulolytic bacterial activity in vitro.

On the other hand, the continuous flow apparatus is more complicated than the all-glass type. Since with in vitro rumen studies it is sometimes necessary to use several fermentation units at one time, the difficulty in using such a system can, therefore, be appreciated.

Microscopic examination of the bacteria which had propagated in 30 hr. showed little difference among the three systems. Occasionally, however, more Gram-positive cells were observed in the semipermeable system.

REFERENCES

(1) Bentley, O. G., Johnson, R. R., Vanecko, S., and Hunt, C. H. Studies on the Factors Needed by Rumen Microorganisms for Cellulose Digestion in Vitro. J. Animal Sci., 13: 581. 1954.

(2) Burroughs, W., Frank, N. A., Gerlaugh, P., and Bethke, R. M. Preliminary Observations upon Factors Influencing Cellulose Digestion by Rumen Microorganisms. J. Nutrition, 40: 9. 1950.

(3) Conway, E. J., and O'Mally, E. Microdiffusion Methods. Ammonia and Urea Using Buffered Absorbents (Revised Methods for Ranges Greater that 10 mg. N). Biochem. J., 36: 655. 1942.

(4) Crampton, E. W., and Maynard, L. A. The Relation of Cellulose and Lignin Content to the Nutritive Value of Animal Feeds. J. Nutrition, 15: 383. 1938.

(5) Dehority, B. A., Bentley, O. G., Johnson, R. R., and Moxon, A. L. Isolation and Identification of Compounds from Autolyzed Yeast, Alfalfa Meal and Casein Hydrolysate with Cellulolytic Factor Activity for Rumen Microorganisms in Vitro. J. Animal Sci., 16: 502. 1957.

(6) Gray, F. V., Pilgrim, A. F., and Weller, R. A. Fermentation in the Rumen of the Sheep. I. The Production of Volatile Fatty Acids and Methane During the Fermentation of Wheaten Hay and Lucerne Hay in Vitro by Microorganisms from the Rumen. J. Exptl. Biol., 28: 76. 1951.

(7) Hershberger, T. V., Bentley, O. G., and Moxon, A. L. A Rapid Volumetric Method for the Determination of Cellulose in Studies with Rumen Microorganisms in Vitro. J. Animal Sci., 14: 725. 1955.

(8) Huntanen, C. N., Saunders, R. K., and Gall, L. S. Fiber Digestion Using the Miniature Artificial Rumen. J. Dairy Sci., 37: 328. 1954.

(9) Johnson, R. R., Dehority, B. A., and Bentley, O. G. Studies on the in Vitro Rumen Procedure: Improved Inoculum Preparation and the Effects of Volatile Fatty Acids on Cellulose Digestion. J. Animal Sci., 17: 841. 1958.

(10) Louw, J. G., Williams, H. H., and Maynard, L. A. A New Method for the Study in Vitro of Rumen Digestion. Science, 110: 478. 1949.

(11) Marston, H. R. The Fermentation of Cellulose in Vitro by Organisms from the Rumen of Sheep. Biochem. J., 42: 564. 1948.

(12) Pennington, R. J. The Metabolism of Short-Chain Fatty Acids in the Sheep. I. Fatty Acid Utilization and Ketone Body Production by Rumen Epithelium and Other Tissues. Biochem. J., 51: 251. 1952.

(13) PENNINGTON, R. J. The Metabolism of Short-Chain Fatty Acids in the Sheep. II. Further Studies with Rumen Epithelium. Biochem. J., 56: 410. 1954.

(14) EL-SHAZLY, K., JOHNSON, R. R., AND DEHORITY, B. A. Biochemical and Microscopic Comparison Between in Vivo and in Vitro Rumen Fermentations. J. Animal Sci., 18: 1518. 1959. (Abstr.)

(15) WARNER, A. C. I. Criteria for Establishing the Validity of in Vitro Studies with Rumen Microorganisms in So-called Artificial Rumen Systems. J. Gen. Microbiol., 14: 733. 1956.

A THEORETICAL RUMEN FERMENTATION BALANCE

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SUMMARY

Certain assumptions are made which permit the calculation of the amounts of CO_2 and CH_4 produced in the rumen which correspond to a given molar distribution of volatile fatty acids present in rumen fluid. This calculation allows the development of a theoretical fermentation balance for a given molar distribution of volatile fatty acids. For a molar distribution of 65 acetate : 20 propionate : 15 butyrate, the following balance is calculated:

$$57.5\ (C_6H_{12}O_6) \rightarrow 65 \text{ acetate} + 20 \text{ propionate} + 15 \text{ butyrate} + 60\ CO_2 + 35\ CH_4 + 25\ H_2O$$

The assumptions upon which the calculations are based are discussed, such as considering volatile fatty acids, CO_2 and CH_4, as sole fermentation products and using the empirical formula $C_6H_{12}O_6$ to represent fermentation substrate. An attempt is made to evaluate the fermentation balance in terms of known experimental data which bear on product-substrate relationships. Values predicted from the theoretical equation are of the same order of magnitude as experimental values.

Application of the balance is made to amplify the interpretation of experimental data concerned with variations in the molar distribution of fatty acids on various dietary regimes. It is proposed that the fermentation balance is useful for interpreting certain quantitative aspects of ruminant nutrition in terms of the quantitative aspects of the rumen microbial fermentation.

Investigations of the microbiology of the rumen and ruminant physiology have provided a qualitative description of the activities of rumen microorganisms and the importance of these activities to the digestion of plant materials by the ruminant. The significance of such microbial activities as plant polysaccharide digestion, volatile fatty acid production, protein digestion and synthesis, and vitamin synthesis is well recognized. Several review articles in this general area are available (3, 6, 15). In the course of preparing some of this material for teaching purposes, the author found it convenient to describe the rumen fermentation in terms of a theoretical fermentation balance. The fermentation balance permits the development of approximations of the quantitative aspects of the rumen fermentation. The theoretical fermentation balance will be the subject of this report.

CALCULATIONS AND DISCUSSION

It should be emphasized that the following analyses are made with idealized systems. Assumptions are made, and a degree of naïveté is introduced, for the sake of achieving coherent, simple descriptions.

Calculation of the balance. For the calculation of the fermentation balance, the following assumptions are made:

(a) The molar proportions of volatile fatty acids found in rumen fluid are 65 acetic : 20 propionic : 15 butyric. These proportions represent the proportions in which these products are produced from fermented substrate.

(b) The only fermentation products produced in addition to the above-mentioned fatty acids are carbon dioxide and methane.

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(c) All fermentation products are formed from plant carbohydrates with the empirical formula $C_6H_{12}O_6$.

Some justification for these assumptions will be presented after the calculation of the model fermentation balance.

An equation for the rumen fermentation can, therefore, be written as:

(1) $X\ (C_6H_{12}O_6) \rightarrow 65\ CH_3CO_2H + 20\ CH_3CH_2CO_2H + 15\ CH_3(CH_2)_2CO_2H + Y\ CO_2 + Z\ CH_4$.

The amounts of CO_2 and CH_4 which would have to be produced to satisfy this equation can be calculated using the standard techniques for calculating fermentation balances (14). The moles of compounds produced containing one carbon atom should be equal to the moles of compounds produced containing two carbon atoms. Butyric acid is assumed to arise from the condensation of two units containing two carbon atoms. Thus:

$$Y\ CO_2 + Z\ CH_4 = 65\ CH_3CO_2H + (2 \times 15\ CH_3(CH_2)_2CO_2H)$$

or (2) $Y + Z = 95$.

Other information exists which will allow the development of an independent equation for the relationship between CO_2 and CH_4. This information arises from the necessity for equivalence between the oxidation level of the fermentation products and the oxidation level of the substrate. The oxidation states are arbitrarily calculated from the following:

$$\text{oxidation state} = \text{number of oxygen atoms per molecule} - \frac{\text{number of hydrogen atoms per molecule}}{2}$$

The oxidation state of the substrate ($C_6H_{12}O_6$) is, therefore, zero and the oxidation states of the products are acetate (0), propionate (−1), butyrate (−2), CO_2 (+2), and CH_4 (−2). The oxidation level is found by multiplying the oxidation state by the moles of product or substrate. The sum of the oxidation levels of the products is equal to zero because the oxidation state of the substrate is zero. Therefore:

$$(65 \times 0) + (20 \times -1) + (15 \times -2) + (Y \times +2) + (Z \times -2) = 0$$

or (3) $Y - Z = 25$.

A simultaneous equation can thus be solved from Equations (2) and (3).

$$\begin{aligned} Y + Z &= 95 \\ Y - Z &= 25 \\ \hline 2Y &= 120,\ Y = 60,\ Z = 35 \end{aligned}$$

Equation (1) can now be written as:

$$X\ (C_6H_{12}O_6) \rightarrow 65 \text{ acetate} + 20 \text{ propionate} + 15 \text{ butyrate} + 60\ CO_2 + 35\ CH_4$$

The number of $C_6H_{12}O_6$ units fermented can be calculated by summing the number of carbon atoms in the products. Equation (1) can now be written as:

(4) $57.5\ (C_6H_{12}O_6) \rightarrow 65 \text{ acetate} + 20 \text{ propionate} + 15 \text{ butyrate} + 60\ CO_2 + 35\ CH_4 + 25\ H_2O$

Discussion of assumptions. The use of the molar proportions of 65 acetic : 20 propionic : 15 butyric for the purposes of calculation is justified on the basis

that these values are not too far astray from many values reported in the literature for the volatile fatty acid composition of rumen fluid. The assumption that the rumen fluid values are indicative of the rates of production of the volatile fatty acids from substrate is supported by the experiments of Carroll and Hungate (4). These investigators followed the rates of production of the acids in rumen fluid immediately after removal of the fluid from the rumen and showed that individual acids were produced in approximately the same proportions as their initial concentrations. Volatile fatty acids, other than those used in these calculations, have been ignored, because they usually do not constitute more than 5% of the volatile fatty acids present. Other products of bacterial fermentations such as hydrogen, ethanol, lactate, succinate, formate, etc. have been ignored, because they usually do not accumulate in significant quantities or they have not been detected in the rumen under normal conditions. Many of these compounds, however, may be intermediates in the formation of the ultimate end products.

The assumption that the fermentation products arise entirely from carbohydrate of empirical formula $C_6H_{12}O_6$ is subject to several criticisms. Certainly, the most important plant carbohydrates digested in the rumen belong to this general class, e.g., cellulose and starch (waters of hydration and dehydration are ignored, since they do not affect the general picture). There are some polysaccharides which contain uronic acids, deoxy sugars, and amino sugars which would alter the calculation of the fermentation balance if they contributed significantly to the fermentation. These carbohydrates, however, are usually only a small part of the ruminant's diet. Noncarbohydrate materials such as proteins and fats may be fermented in the rumen, but fats are not very important in ruminant diets and proteins must, for the most part, be conserved as ingested protein per se, or as microbial protein, in order for the ruminant to be able to survive. There may be certain situations in which the net[1] protein fermentation could be relatively large. It is known that large amounts of NH_3 accumulate in the rumen if the ruminant is fed protein without an adequate supply of carbohydrate (5). It is the author's opinion that in most situations the net protein fermentation is small relative to the carbohydrate fermentation. This is supported by the fact that the ruminant nitrogen requirement is not greater than the nonruminant requirement (13).

Xylans and other pentosans would probably be fermented by a mechanism involving cleavage into a two-carbon and a three-carbon compound. The two-carbon unit would not be accompanied by a one-carbon unit. Thus, the calculations equating one-carbon units with two-carbon units would have to be corrected for the amount of two-carbon units arising from pentase cleavage. These corrections have not been applied to the fermentation balances calcu-

[1] It is conceivable that gross protein fermentation always occurs and is considerable in magnitude. If this gross fermentation is balanced by resynthesis of microbial protein, the contribution to the over-all fermentation would be minor. Such a situation is comparable to ordinary turnover of protein, except that the rumen turnover involves the breakdown of ingested protein and the resynthesis of microbial protein. Net fermentation would occur when the rate of breakdown of protein exceeds the rates of resynthesis.

lated in this paper and it is assumed that all products arise from the cleavage of hexose units.

Evaluation of the balance. One question which is immediately raised about the fermentation balance is whether it is a realistic assessment of the in vivo rumen fermentation. Unfortunately, there are not too many measurements available which will allow a critical evaluation. A few indirect tests can be made, however, which do supply a small degree of encouragement.

One test which can be applied derives from measurements which have been made of the amount of methane produced in the rumen per amount of digestible carbohydrate fed. Several values have appeared in the literature for this relationship and they are approximately in the same range. Kellner (9) reported an increase of 4.29 g. of methane for every 100 g. of digestible carbohydrate fed above the maintenance level. In similar types of experiments, Armsby and Fries (1) reported a figure of 4.8 and 4.7 g. of methane for every 100 g. of digested carbohydrates fed as roughages or a mixture of roughages and concentrates, respectively. For 100 g. of digested carbohydrate in Sudan hay added to a maintenance ration of Sudan hay, 4.4 g. of methane were produced [Kleiber *et al.* (12)]. It can be calculated from the model equation that 6.0 g. of methane would be produced from 100 g. of pure cellulose digested (57.5×162 = grams cellulose). Thus, the calculated value for methane production per 100 g. of carbohydrate digested is anywhere from 25 to 40% higher than the experimentally determined values. Possible explanations for the discrepancy between the theoretical and experimental values will be discussed after further comparisons of experimental results with calculated results.

Another comparison can be made with a value of 3.12 meq. of volatile acid produced from 430 mg. of cellulose digested by rumen fluid, reported by Carroll and Hungate (4). The theoretical equation predicts that the volatile acid produced from 430 mg. of cellulose should amount to 4.62 meq. The calculated value for acid production is 48% higher than the experimental value.

Another comparison with experimental values can be made on the basis of the rates of production of volatile fatty acids found by Carroll and Hungate (4) by in vitro measurements in rumen fluid of steers on different diets. These authors reported figures of 1.42 and 2.35 meq. of volatile acid produced per 100 g. of rumen fluid per hour for hay and grain diets, respectively. By taking the ratio of methane produced to volatile fatty acids produced from the theoretical equation, and extrapolating to a 24-hr. period and 70 kg. of rumen contents, a theoretical yield of methane can be estimated. The calculated values are 187 liters of methane for the hay diet and 312 liters for the grain diet. Kleiber *et al.* (12) experimentally measured a daily rate of methane production of 180 liters per day for dry cows on maintenance rations and 280 liters per day for lactating cows on production diets.

It should also be noted that the molar percentage composition of rumen gas from cows on alfalfa pasture has been reported as 67% CO_2; 26% CH_4; 7% $N_2 + H_2$; 0.1% H_2S, and less than 1% O_2 by Kleiber *et al.* (11). Similar results had been obtained earlier by Tappeiner (16). If we use the figure of 93% from the data above, for the total contribution of CO_2 and CH_4 to the

rumen gas, the percentages of CO_2 and CH_4 predicted from Equation (4) would be 59 and 34%, respectively. Kleiber (10) has pointed out that saliva can contribute to the total rumen CO_2 only when the rumen pH drops below 6.9. Below pH 6.9, the tendency would be to raise the proportion of CO_2 in the rumen gas as a result of the acidification of the bicarbonate in solution by acid fermentation end products.

The results of the comparisons between the experimental and theoretical values indicate that the predictions of the theoretical equation are at least of the same order of magnitude as experimental values. The experiments with which the comparisons are made were obviously not designed to check the fermentation balance, and certain critical information remains unknown for each particular experiment. It is interesting to note that in the two cases where the comparison is made on a product-to-substrate ratio comparison, i.e., CH_4 : carbohydrate or volatile fatty acid : carbohydrate, the amounts of product predicted from the theoretical equation tend to be higher than those amounts actually found. It is not very useful to comment on the comparison of theoretical and experimental total methane production per day, because the theoretical estimation involves assumptions concerning rates of production and volumes of rumen ingesta which are difficult to evaluate.

Applications of the balance. A useful feature of any theory is that it provides a base for the discussion of the various parameters which the theory embodies, whether the theory be correct or incorrect. We might consider, for example, some possible reasons for the discrepancies between the theoretical and experimental values found in the product-substrate ratio comparisons. One possible explanation is that the products considered, i.e., acetate, propionate, butyrate, CH_4, and CO_2, do not represent all of the products formed from ingested carbohydrate. Thus, more carbohydrate should be digested per unit of the above end products formed. Such a situation would arise if a large amount of ingested carbohydrate is incorporated into microbial carbohydrate. Another possibility is the formation of a product which diffuses rapidly through the rumen wall, so that it is not normally found in the rumen, e.g., lactate, ethanol, succinate, and formate. It should be pointed out that, except for lactate, such products would disturb the calculated CO_2 and CH_4 values, because they would affect the predicted amounts of one-carbon compounds and the predicted oxidation-reduction balance of the products. Lactate would have no effect, because its oxidation state is zero and it is a three-carbon compound.

Calculation of a theoretical equation is useful in its emphasis of the interrelationships of end products. It is of interest to note the consequences of a change in the proportion of volatile fatty acids. An increase in the proportions of propionic and butyric acids and a decrease in acetic acid would tend to decrease the methane yield. Calculations of fermentation balances from the data of Elliot and Loosli (7), concerning the molar proportions of volatile fatty acids on different dietary regimes, show a decrease of approximately 9% in the methane produced per mole of carbohydrate digested when the cows were fed 20% of their estimated net energy (ENE) as roughage, as compared to 60%. The calculated values for CH_4 and CO_2 are shown with the experi-

mental values in Table 1, along with the calculated amount of carbohydrate fermented to these products (calculated as starch). Valerate is assumed to be synthesized by a condensation of a two-carbon unit with a three-carbon unit. Calculations of the heats of combustion of the fatty acid products and the substrate fermented (the heat of combustion of starch was used for the calculations) show little difference in the therms of total volatile fatty acids produced per therm of digested carbohydrate for the 20 and 60% ENE as roughage diets. It is interesting to note that the fermentation balance indicates no difference in the available therms of propionic acid per therm of substrate fermented on the two diets, whereas the available therms of butyrate per therm of substrate increases on the low roughage diet. The order of magnitude of the increase, however, can not explain the increased efficiency of the low roughage diet.

TABLE 1
Fermentation end products from data of Elliot and Loosli (7)

Diet	Moles per 100 moles VFA						
	Starch fermented	Acetate	Propionate	Butyrate	Valerate	CH_4	CO_2
High roughage	57.3	66.6	18.7	11.8	2.9	33.9	59.5
Low roughage	59.1	60.5	21.4	15.4	2.7	31.9	62.1

Another point of interest from the above work is that the net efficiency of the fermentation calculated from the balance and represented as therms of available fatty acids to therms of carbohydrate fermented is 0.71 and 0.72 for the high and low roughage diets, respectively. Using the heat of combustion figure of 0.340 therm per pound of fat-corrected milk (2), the efficiencies of the conversion of digestible energy to milk energy, in terms of therms of milk produced per therm of digestible energy consumed over and above maintenance requirements, are 0.53 and 0.66 for the high and low roughage diets, respectively.[2] If it is assumed that the fatty acids derived from the digestible energy are the source of energy for milk production, the efficiency of milk formation can be calculated in terms of the therms of fatty acids produced from the digestible energy utilized. The therms of fatty acids produced per therm of digestible energy consumed can be approximated from the fermentation balances by the previously mentioned values of 0.71 and 0.72 therms of available fatty acid per therm of carbohydrate fermented for the high and low roughage diets, respectively. The efficiency of the conversion of the available fatty acid energy to milk energy would then be 75% for the high roughage diet and 92% for the low roughage diet. In other words, approximately 17% more of the energy available as fatty acids is used for milk production when the low roughage ration is supplied instead of the high roughage ration.

In contrast to the fermentation balances based on the work of Elliot and Loosli, fermentation balances based on the work of Eusebio *et al.* (8) suggest that significantly different energy yields could be obtained on high corn or

[2] Estimated from Figure 2 of Elliot and Loosli (7).

flaked corn diets in contrast to hay-corn rations. These workers measured the volatile fatty acid concentration of rumen fluid on various dietary regimes. If one calculates fermentation balances from the volatile fatty acid concentration[3] for the alfalfa hay-corn meal diet (12.9 lb. U. S. No. 2 alfalfa hay and 5.7 lb. corn meal fed daily) and for the corn meal diet (11.8 lb. corn meal fed daily), the energy yield in therms of fatty acids produced from a therm of substrate is about 13% greater for the corn meal diet than for the hay–corn meal diet. The calculated values for CH_4, CO_2, and starch are shown with the experimental values for volatile fatty acids in Table 2. For the alfalfa hay–corn meal ration the fatty acids represent 0.75 therm per therm of carbohydrate, whereas 0.85 therm is contained in the fatty acids produced on the corn meal ration.

TABLE 2

Fermentation end products from data of Eusebio *et al.* (8)

Diet	Moles per 100 moles VFA						
	Starch fermented	Acetate	Propionate	Butyrate	Valerate	CH_4	CO_2
Corn meal	54.5	38	43	11	3	13	50
Alfalfa hay–corn meal	57	56	24	14	3	28	59

These calculations serve to emphasize the quantitative relationships between end products and substrate and between end products themselves. The exact numerical values of these relationships are not known, and the calculations are presented as models which may be useful for teaching purposes. Further research in the area of ruminant microbiology and physiology may provide a more accurate picture of the rumen fermentation in quantitative terms. Studies of product-substrates relationships and the rates of production of products from substrates may allow a quantitative description of the nutrition of the ruminant in terms of the quantitative aspects of the microbial fermentation.

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REFERENCES

(1) Armsby, H. P., and Fries, J. A. Net Energy Values of Feeding Stuffs for Cattle. J. Agr. Research, 3: 435. 1915.
(2) Brody, S. Bioenergetics and Growth. Reinhold Publ. Corp., New York. 1945.
(3) Bryant, M. P. Bacterial Species of the Rumen. Bacteriol. Revs., 23: 125. 1959.
(4) Carroll, E. J., and Hungate, R. E. The Magnitude of the Microbial Fermentation in the Bovine Rumen. J. Appl. Microbiol., 2: 205. 1954.
(5) Chalmers, Margaret I., and Synge, R. L. M. Protein Digestion in Ruminants. Advances in Protein Chemistry, 9: 98. 1954. Academic Press, New York.
(6) Doetsch, R. N., and Robinson, R. Q. The Bacteriology of the Bovine Rumen: A Review. J. Dairy Sci., 36: 115. 1953.

[3] The C_6+ fatty acids were ignored for calculation purposes.

(7) ELLIOT, J. M., AND LOOSLI, J. K. Relationship of Milk Production Efficieney to the Relative Proportions of the Rumen Volatile Fatty Acids. J. Dairy Sci., 42: 843. 1959.
(8) EUSEBIO, A. N., SHAW, J. C., LEFFEL, E. C., LAKSHAMANAN, S., AND DOETSCH, R. N. Effect on Rumen Volatile Fatty Acids and Rumen Microbial Dissimilation of Glucose-C^{14} of Corn Meal when Fed Exclusively and in Combination with Hay or Certain Additives. J. Dairy Sci., 42: 692. 1959.
(9) KELLNER, O. Die Ernahrung der Landwirtschaftlichen nutztiere. 8th ed. Paul Parey, Berlin. 1919.
(10) KLEIBER, M. A Review of Bloat in Ruminants. National Research Council, Publ. 388. Washington, D. C. 1956.
(11) KLEIBER, M., COLE, H. H., AND MEAD, S. W. Bloat in Cattle and Composition of Rumen Gases. J. Dairy Sci., 26: 929. 1943.
(12) KLEIBER, M., REGAN, W. M., AND MEAD, S. W. Measuring Food Values with Dairy Cows. Hilgardia, 16: 511. 1945.
(13) MORRISON, F. B. Feeds and Feeding. 22nd ed. Morrison Publ. Co., Ithaca, New York. 1957.
(14) NEISH, A. C. Analytical Methods for Bacterial Fermentations. National Research Council of Canada, Rept. No. 46-8-3 (Rev.). Saskatoon.
(15) OXFORD, A. E. The Bacteriology and Protozoology of Ruminant Digestion. J. Sci. Food Agr., 6: 413. 1955.
(16) TAPPEINER, H. Die Darmgase der Pflanzen fresser. Deut. Chem. Ber., 14: 2375. 1881.

SOME EFFECTS OF A SUPPLEMENTARY CELLULASE PREPARATION ON FEED UTILIZATION BY RUMINANTS [1]

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SUMMARY

Enzyme 19AP, a fungal cellulase, added to rumen fluid greatly enhanced cellulolytic activity as measured by change in viscosity of sodium carboxymethylcellulose solution.

Thirty dairy calves were used to test the effect of the enzyme on feed utilization in a growth trial. Enzyme 19AP exhibited no effect on feed utilization as measured by average daily gain of calves fed at the same rate.

In a digestion trial, eight sheep were given basal diets or autoclaved basal diets with and without 500 mg. of Enzyme 19AP twice daily in two 4×4 Latin-square designs. Enzyme 19AP exhibited no effect on apparent digestibility of ration constituents. Destruction of the cellulolytic activity associated with the feed by autoclaving had no effect on the apparent digestibility of cellulose. However, dry matter and protein digestibilities were significantly lowered in the autoclaved diets.

Enzymes produced by the digestive tract of ruminants are unable to digest cellulose. However, large amounts of cellulose in the feeds utilized by ruminants are broken down by the enzymatic action of the rumen microflora. This digestive process not only degrades the fibrous material, but also releases the nutrients contained inside the cells so they can be more easily attacked by the animal's digestive juices.

The dependence of ruminants on symbiotic bacteria for roughage utilization suggests that addition of a supplementary cellulase to the rumen might be beneficial either by increasing the rate or the completeness of cellulose degradation.

According to Siu (9), commercial enzyme preparations, presumably cellulolytic, have been sold as digestive aids. Siu also cites the report by Rosenkilde and Bogger, that the digestibility of cellulose by sows was increased from 50 to 90% by inoculating the feed with cellulolytic bacteria.

Enzyme 19AP [2] was claimed to be highly active on carboxymethylcellulose (CMC) solution, as measured by change in viscosity and the amount of reducing sugars produced when CMC is hydrolyzed. However, many noncellulolytic organisms can hydrolyze soluble cellulose derivatives (9). Possible improvement in feed utilization from supplementing normal ruminant diets with such an enzyme needed investigation. Results of providing Enzyme 19AP on growth of young dairy calves and on digestibility of rations by sheep are reported herein. Since water washings of hays were found to have cellulolytic activity

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[2] A commercial enzyme preparation from *Aspergillus niger* generously supplied for these trials by Rohm and Haas Company, Philadelphia, Pennsylvania.

(5), the effects of this enzyme source on digestibility were tested by feeding rations in which the cellulolytic activity of the feed was destroyed by autoclaving.

EXPERIMENTAL PROCEDURE

The ability of Enzyme 19AP to supplement the cellulolytic activity of rumen fluid following in vitro and in vivo addition was tested by enzymatic assay. Possible in vivo effects were further evaluated in a growth trial with dairy calves and in a digestion trial with sheep.

Enzymatic assay. Enzyme 19AP was added to rumen fluid to determine the ability to supplement the cellulolytic activity of rumen fluid as measured by a change in viscosity of CMC. Rumen fluid, collected from fistulated cows, was filtered through four layers of cheese cloth and centrifuged at 1,000 r.p.m. for 2 min. to remove the large feed particles. Two grams of Enzyme 19AP were mixed with 100 ml. of rumen fluid in a Waring blender, and dilutions were made with rumen fluid to give 2, 1, 0.1, 0.01, and 0.001% concentrations (w/v) of Enzyme 19AP. Rumen fluid without Enzyme 19AP was used as a control. This experiment was replicated with rumen fluid obtained on three different days.

Samples of rumen fluid from calves on the growth trial and from the sheep on the digestion trial were assayed while the trials were in progress.

The procedure of Bell *et al.* (2), as adapted by Cason and Thomas (4), was used to measure the cellulolytic activity of rumen fluid. Five milliliters of a 1.2% solution of sodium-carboxymethylcellulose 70M in a citrate buffer (pH 4.9-5.0) was placed in an Ostwald-Fenske viscosity pipette suspended in a water bath at 37° C. One milliliter of the rumen fluid to be assayed for cellulolytic activity was added to the CMC solution and mixed thoroughly by drawing air through the mixture with vacuum applied to the large opening of the viscosity pipette. The initial flow time was determined immediately and subsequent determinations were made until there was a 50% decrease in specific flow time. The number of minutes required for this decrease was used to compare the cellulolytic activity of the different rumen fluids.

Growth trial. Thirty dairy calves in two age groups were assigned to trios on the basis of breed, weight, and age. The younger group consisted of five trios of male Holstein calves approximately 8 wk. of age. The older group consisted of two trios of Holstein heifers and one each of Guernsey, Jersey, and Ayrshire heifers. These older calves ranged from 11 to 32 wk. of age. Initial weights for all groups are given in Table 1. The grain mixture averaged 13.6% crude protein and was limited to 2.4 lb. per day for the younger calves and 3.0 lb. per day for the older calves. The younger calves were fed U.S. No. 2 alfalfa light grass mixed hay and the older calves were fed a U.S. No. 2 orchard grass hay. Each hay was fed chopped and the amounts allowed were constant within each trio. Both hay and grain were fed twice daily.

One calf in each trio served as a control and was fed as outlined above. One gram of Enzyme 19AP was administered twice daily to each of the two other calves in the trio. One received the enzyme via gelatin capsule and the

other had it mixed into the grain to allow a more even distribution in the rumen. A 2-wk. standardizing period preceded the 8-wk. comparison period in which the enzyme was administered.

One weight for younger calves and an average of three consecutive daily weights for older calves, taken at the end of the standardizing and comparison periods, were used to calculate an average daily gain for each calf.

The cellulolytic activity of the rumen fluid of these calves was determined by the procedure previously described. Samples of rumen fluid were obtained by stomach tube from each calf in the 2nd wk. of the standardizing period, in the 4th wk. of the comparison period for older calves, and in the 8th wk. for the younger group.

Digestion trial. Two squares of a 4×4 Latin-square trial (6) were used. Four similar polled Dorset rams, averaging 71 lb., were randomly assigned to Square I. Four crossbred wethers, averaging 76 lb., were randomly assigned to Square II. Each 2-wk. period in the design consisted of 1 wk. for standardization and 1 wk. for collection of feces. The rations were basal, basal plus Enzyme 19AP, autoclaved basal, and autoclaved basal plus Enzyme 19AP. Enzyme 19AP (500 mg. twice daily) was administered in gelatin capsules prior to each feeding.

The basal diet was alfalfa hay for Square I and orchard grass hay supplemented with 1 lb. of soybean meal to 4 lb. of hay for Square II. Four per cent crude cottonseed oil was added to the orchard grass hay diet to partially eliminate the dust. The hays for the autoclaved diets were autoclaved at 15 p.s.i. for 20 min. to destroy the enzymatic activity associated with the feed. Each sheep received 1.0 lb. of feed twice daily, except that one Dorset ram assigned to Square I received 0.7 lb. twice daily in each period.

The sheep were weighed at the start of the trial and at the end of each period. Samples of rumen fluid were taken by stomach tube on the last day of each period and their cellulolytic activity, together with those of the hays, was measured as previously outlined.

Feed, weigh-backs, and fecal samples were analyzed for proximate constituents by the A.O.A.C. methods (1), and nitrogen-free extract (N.F.E.) was calculated. Cellulose was determined by the method of Matrone (7).

RESULTS AND DISCUSSION

Enzymatic assay. Rumen fluid alone required an average of 75.0 min. to decrease the specific flow time of CMC 50%; whereas, rumen fluid with 0.001, 0.01, 0.1, 1.0, and 2.0% Enzyme 19AP required an average of 68.7, 36.0, 13.7, 2.7, and 3.3 min. ($\pm$ 4.39) to decrease the specific flow time. Expressing the activity as the number of minutes necessary for a 50% decrease results in a smaller number of minutes, representing a greater activity.

This highly significant ($P < 0.01$) increase in cellulolytic activity resulting from the addition of enzyme was of smaller magnitude at the higher enzyme concentrations. The marked effect of Enzyme 19AP on the cellulolytic activity, as measured herein, confirms the claims of the manufacturer.

Growth trial. The results are given in Table 1. No appreciable differences existed among the treatment groups in feed consumed. The administration of cellulase enzyme was without effect on feed utilization, as measured by rate of gain of dairy calves.

TABLE 1
Mean initial weight, daily feed consumption, and weight gains for calves in the growth trial

Criterion	Age group	Control	Enzyme 19AP	
			Via capsule	In grain
		(lb.)		
Standardizing period				
Initial weight	Younger	136	153	165
	Older	257	261	276
Comparison period				
Feed consumption	Younger	2.2	2.2	2.2
Concentrate	Older	3.0	3.0	3.0
Hay	Younger	3.3	3.5	3.9
	Older	3.6	3.6	3.6
Weight gain	Younger	1.37	1.28	1.34
	Older	0.74	0.69	0.74
	All calves	1.06	0.98	1.04

The younger calves gained 1.33 lb. per day, which was significantly greater than the 0.72 lb. gained by the older group. The poorer quality of hay, the limited amount of grain, and the higher maintenance requirements of the older calves probably contributed to this lesser gain by the older group. There was no suggestion of a different effect of the enzyme in the two age groups.

Although Enzyme 19AP added to fresh rumen fluid in the laboratory increased the activity markedly, feeding the enzyme to calves had no measurable effect on the cellulolytic activity of their rumen fluid. Increased activity was expected, a priori, because the amount of enzyme fed based on calculated dilution in the rumen was adequate to effect a substantial response. Factors responsible for the difference in the ability of Enzyme 19AP to increase cellulolytic activity in vitro and in vivo were not determined.

Digestion trial. The apparent digestibility values are reported in Table 2. Because no square-by-treatment interaction was observed, the ration means given are averaged over both squares (basal diets). The digestion coefficients

TABLE 2
Mean apparent digestibility of ration constituents

Constituent	Ration				Standard error of a mean
	Basal		Autoclaved		
	No enzyme	Enzyme	No enzyme	Enzyme	
	(%)				
Dry matter	65.31	65.13	64.19	62.25	0.80
Cellulose	66.56	66.49	67.75	65.88	0.92
Crude fiber	59.29	59.52	58.97	56.61	1.43
N.F.E.	67.26	66.64	67.62	65.57	0.88
Crule protein	75.47	74.74	67.68	66.82	0.93

for cellulose, crude fiber, and NFE, and the total grams of cellulose and crude fiber digested, were not statistically different for the four treatments. Although Enzyme 19AP can hydrolyze altered cellulose in vitro (10), it did not increase apparent digestibility as measured in this trial.

Autoclaving the hays essentially eliminated the cellulolytic activity associated with them, but this treatment had no measurable effect on the apparent digestibility of the cellulose. This finding is in agreement with the report by Bentley and Moxon (3), that the activity in forage did not contribute materially to the total cellulolytic activity in artificial rumen flasks.

Autoclaving did significantly lower the digestibility of both dry matter (2.00%) and crude protein (7.86%). This protein effect with ruminants appears to be similar to that observed previously for heat-treated protein fed to rats (8). The average crude protein contents of the diets were not affected by autoclaving.

The average weight gains per 14-day period of 0.9, 2.0, 2.1, and 0.4 lb. for the basal, basal plus enzyme, autoclaved basal, and autoclaved basal plus enzyme, respectively, were not significantly different.

There was no significant difference among the cellulolytic activity of the rumen fluids in the enzymatic assay. As in the growth trial, other factors presumably affected the activity more than did added enzyme or the activity of the feed.

REFERENCES

(1) Association of Official Agricultural Chemists. Official Methods of Analysis. 6th ed. College Offset Press, Philadelphia. 1950.

(2) Bell, T. A., Etchells, J. L., and Jones, I. D. A Method for Testing Cucumber Salt-Stock-Brine for Softening Activity. ARS-72-5. USDA. 1955.

(3) Bentley, O. G., and Moxon, A. L. The Cellulolytic Activity of Organisms Associated with Dried Forages. J. Animal Sci., 15: 1257. 1956.

(4) Cason, J. L., and Thomas, W. E. Cellulolytic Activity of Bovine Rumen Liquid upon a Soluble Cellulose Derivative. J. Dairy Sci., 38: 608. 1955.

(5) Cason, J. L., Mochrie, R. D., and Thomas, W. E. Self Containing Cellulose Digestors of Common Ruminant Feeds. J. Animal Sci., 14: 1236. 1955.

(6) Cochran, W. G., and Cox, G. M. Experimental Design. John Wiley & Sons, Inc., New York. 1950.

(7) Matrone, G. A Study of Lignin and Cellulose Methods for the Chemical Evaluation of Feeds. M.S. thesis. Cornell University, Ithaca, New York.

(8) Mitchell, H. H., Hamilton, T. S., and Beedles, Jessie R. The Nutritional Effects of Heat on Food Proteins, with Particular Reference to Commercial Processing and Home Cooking. J. Nutrition, 39: 413. 1949.

(9) Siu, R. G. H. Microbial Decomposition of Cellulose. Reinhold Publishing Corporation, New York. 1951.

(10) Walseth, C. S. The Influence of the Fine Structure of Cellulose on the Action of Cellulases. Tappi, 35: 233. 1952.

CORRECTION FOR HEMOLYSIS IN A PHOTOMETRIC METHOD FOR ASSAYING SULFOBROMOPHTHALEIN IN THE BLOOD OF CATTLE IN TESTS FOR HEPATIC FUNCTION[1]

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SUMMARY

Varying degrees of hemolysis of blood have interfered with photometric measurements of the dye sulfobromophthalein (Bromsulphalein or BSP) in studies of hepatic function. In the usual procedures plasma blanks are measured in acidic media, since dye colors are developed in basic media. However, the optical density (O.D.) of hemoglobin (as hematin), when measured in an acidic medium, differs from that obtained in a basic medium. Similarly, varying degrees of hemolysis as between plasma blank (no dye) and plasma dye tubes do not allow proper blank correction when either acidic or basic plasma blanks are used.

In developing the improved method, plasma blank O.D. of a series of blood samples containing no dye, but with varying degrees of induced hemolysis, were measured both in acidic and in basic media. Regression equations were derived to predict the basic plasma blank O.D. from acidic plasma blank O.D. When such predicted basic plasma blank O.D. readings were applied to plasma samples containing known amounts of dye but varying amounts of hematin, accurate estimates of dye contents were obtained. Also, when the improved photometric method was used in performing actual hepatic function tests in calves and cows, marked improvement resulted in the precision or repeatability of the measures as compared to the usual procedures.

It is suggested that this new procedure for photometric analysis be used only in those instances where hemolyzed plasma samples are present in the series of samples to be analyzed.

Photometric procedures described for the assay of sulfobromophthalein (BSP or Bromsulphalein[3]) in blood serum or plasma (1, 2, 4, 5, 6) have been found to be inaccurate when applied to blood samples exhibiting varying degrees of hemolysis. If a split plasma sample containing hemoglobin is used to prepare an acidic plasma blank for a basic dye tube, the hematin thus formed will have a different optical density in the acidic and basic tubes and will cause an inaccurate dye optical density value. Similarly, if a basic plasma sample containing no dye is used as a blank for a basic dye-containing plasma sample, differing degrees of hemolysis in the two plasma fractions will also lead to inaccurate dye optical density values.

The idea for the improved method reported here originated from the alkali hematin method of Wu (3), in which hemoglobin is first treated with acid to form acid hematin and is then treated with alkali to form alkali hematin. Basi-

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cally, the modification involved the development of a regression equation whereby basic plasma blank optical density values were predicted from the corresponding acidic plasma blank optical density values. The optical densities of a given dye-containing plasma sample were determined serially in acidic and the basic media. The predicted basic plasma blank optical density was used as a plasma blank.

METHODS AND PROCEDURES

Photometric procedure. The photometric procedure for the assay of sulfobromophthalein sodium in plasma was modified slightly from that reported by Mixner and Robertson (6). Using round colorimeter tubes (11 mm. i.d.), to 1.0 ml. of plasma were added 3.0 ml. of water and 1.0 ml. of 0.5 *N* HCl. This was allowed to stand for 10 min. to develop the acid hematin (if present). The optical density (O.D.) of the tube was then read in a spectrophotometer at 580 mμ. Immediately after this reading, 0.5 ml. of 2.5 *N* NaOH was added to the tube and mixed by swirling. The O.D. of the tube was read a second time at 580 mμ.

Development of regression equation. To determine the relationship of the O.D. of hemolyzed plasma in acidic and basic media, large blood samples were obtained from four cows. Each blood sample was divided in half. One fraction was centrifuged to produce clear (nonhemolyzed plasma); the second fraction was treated with a small amount of ether prior to centrifugation to induce hemolysis roughly equivalent to that found in a severely hemolyzed blood sample normally collected. The clear and hemolyzed plasmas were mixed in varying ratios to produce a series of seven plasma subsamples with increasing amounts of hemolysis (total of 28 plasma subsamples).

Each of the plasma subsamples was subjected to the photometric procedure previously referred to, to obtain an acidic plasma blank O.D. and a basic plasma blank O.D. for each plasma subsample.

Regressions of the base O.D. readings on the acid O.D. readings were calculated on a within-plasma sample basis.

Test of the accuracy of the new photometric method. Blood samples were collected from four cows and each divided and treated as above so as to produce both clear and hemolyzed plasmas. To the four possible combinations of clear and hemolyzed plasmas was added either 0.03 or 0.015 mg. of sulfobromophthalein to simulate those levels of dye generally encountered in the 5- and 10-min. plasma samples in an actual test of hepatic function as follows:

A, Tube 1—no hemolysis, 0.03 mg. BSP; Tube 2—no hemolysis, 0.015 mg. BSP.
B, Tube 1—no hemolysis, 0.03 mg. BSP; Tube 2—hemolysis, 0.015 mg. BSP.
C, Tube 1—hemolysis, 0.03 mg. BSP; Tube 2—no hemolysis, 0.015 mg. BSP.
D, Tube 1—hemolysis, 0.03 mg. BSP; Tube 2—hemolysis, 0.015 mg. BSP.

Upon running these pairs of tubes through the new photometric procedure (each tube as own blank), BSP fractional clearance values (regression coefficients) were calculated.

A similar series of tubes was run using the original method of plasma blank correction (5).

All BSP fractional clearance values obtained by the two methods of blank correction were compared with a calculated theoretical BSP fractional clearance value or regression coefficient.

Repeatability and precision of BSP clearance measures. BSP liver function tests were run in four bull calves and four lactating cows, to measure certain aspects of the repeatability and precision of the new photometric procedure as compared to the old procedure. The tests were performed on two successive days. On each day four samples of blood were taken per test, two in succession at the 5-min. period and two again at the 10-min. period. The first samples taken at each time interval were considered as a pair for calculation of BSP fractional clearance. Each blood sample was divided in the laboratory and separate plasmas were obtained from each, allowing the calculation of sub-BSP fractional clearance values.

Before the development of the improved photometric method, data were being accumulated for normal standards, repeatability, and precision of BSP clearance measures, using the older photometric method. Tests were performed on two successive days on a total of ten bull calves and 11 cows. These data are compared with those obtained by use of the improved photometric method.

RESULTS

Development of regression equation. The four plasma samples (each with a series of seven subsamples having varying degrees of hemolysis) were subjected to the photometric procedure described, to obtain corresponding acidic and basic plasma blanks O.D. readings. An analysis of covariance indicated that there were no significant differences among the regression coefficients of basic plasma blank O.D. on acidic plasma blank O.D. for four plasma samples. Accordingly, a regression equation on the pooled data on a within-plasma sample basis was calculated by the method of least squares as being the most suitable equation.

The regression equation developed was $Y = 1.872\ X - 0.0333$, in which Y equaled the O.D. of basic plasma blank and X equaled the O.D. of the acidic plasma blank. The coefficient of correlation between the variables was +0.999. Thus, in the following example, the acidic plasma blank O.D. of 0.03 mg. of BSP was 0.118. Substituting in the regression equation, the corresponding predicted basic plasma blank O.D. was 0.187. After adding excess base to develop dye color, the O.D. for the sample was 0.796 and by subtracting the predicted basic plasma O.D. from this, an adjusted dye O.D. value of 0.609 was obtained.

	Plasma blank O.D.		BSP plasma O.D.	
BSP *(mg.)*	Acidic	Basic	Excess base	Adjusted
0.03	0.118	0.187	0.796	0.609

Test of the accuracy of the new photometric method. The simulated BSP fractional clearance values as calculated in the various combinations of clear and hemolyzed subplasmas of the four plasma samples are shown in Table 1. The marked improvement in the accuracy of the simulated BSP fractional

TABLE 1

Comparison of accuracy of original and improved photometric methods as used to determine simulated BSP fractional clearance values (four plasma samples)

Photometric method	Characteristic	Simulated BSP fractional clearance values			
		Plasma tube hemolysis combinations			
		A	B	C	D
Original	BSP fractional clearance				
	Theoretical mean	0.1387	0.1387	0.1387	0.1387
	Observed mean	0.1419	0.0404	0.1934	0.0971
	Deviations from theoretical[b]	0.0050	0.1117	0.0623	0.0473
	Deviations from theoretical as per cent of theoretical[c]	3.60	80.53	44.92	34.10
Improved	BSP fractional clearance				
	Theoretical mean	0.1387	0.1387	0.1387	0.1387
	Observed mean	0.1351	0.1366	0.1376	0.1364
	Deviations from theoretical[b]	0.0077	0.0075	0.0098	0.0082
	Deviations from theoretical as per cent of theoretical[c]	5.55	5.41	7.07	5.91

[a] A 1st plasma tube clear; 2nd plasma tube clear.
B 1st plasma tube clear; 2nd plasma tube hemolyzed.
C 1st plasma tube hemolyzed; 2nd plasma tube clear.
D 1st plasma tube hemolyzed; 2nd plasma tube hemolyzed.

[b] Calculated as $\sqrt{\frac{\Sigma (X_o - X_t)^2}{N-1}}$ where X_o and X_t equaled the observed and theoretical mean BSP fractional clearance values, respectively. This is equivalent to a standard error of a single determination.

[c] This is equivalent to a coefficient of variation of a single determination.

clearance values by the use of the improved photometric procedure was readily apparent. An analysis of variance indicated that the difference between photometric methods was highly significant. Differences among the plasma hemolysis combinations were significant only for the old photometric procedure.

Repeatability and precision of BSP clearance measures. Marked differences were found between calves and cows with respect to the various BSP clearance measures and, hence, the data were analyzed separately. Table 2 presents the estimated variances and the variance percentages for the several sources of variation of the BSP clearance measures. The animal variance percentages are the repeatability estimates for the BSP clearance measures. In general, the repeatability estimates of the BSP clearance measures are higher in the cows than in the calves. However, the repeatability of BSP volume clearance as milliliters of plasma cleared of BSP per minute per kilogram of body weight was satisfactorily high in both calves (89.8%) and cows (98.5%).

The precision of making BSP clearance measures in calves and cows is perhaps best shown by use of standard errors and the coefficient of variations of single determinations. These are shown in Table 3, in which the measures are based on the square roots of the sum of the variances for days in animal, samples in days in animal, and duplicates in samples in days in animals as taken from Table 2. In all instances, the standard errors and coefficients of variation of single determinations were correspondingly lower with the improved than with the original photometric method.

TABLE 2

Estimated variances and variance percentages for BSP clearance measures using the improved photometric procedure

Source of variance	Degrees of freedom	BSP fractional clearance		Plasma volume, per cent of body weight		Blood volume, per cent of body weight		BSP plasma volume clearance, ml/min/kg B.W.	
		Variance	Variance per cent	Variance	Variance per cent	Variance	Variance per cent	Variance	Variance per cent
Bull calves									
Animals	3	.0005227	56.8	1.233	39.1	7.130	49.2	13.6521**	89.8
Days: A	4	.0002253*	24.5	1.163**	36.8	4.714*	32.5	1.0772*	7.1
Samples: D: A	8	.0000998*	10.8	0.522**	16.5	1.922*	13.3	0.3247**	2.2
Duplicates: S: D: A	16	.0000725	7.9	0.240	7.6	0.732	5.0	0.1434	0.9
Total	31	.0009203	100.0	3.158	100.0	14.498	100.0	15.1975	100.0
Cows									
Animals	3	.0031243**	95.6	0.524**	92.6	0.830**	89.2	10.0802**	98.5
Days: A	4	.0000813*	2.5	−0.005	−0.9	−0.036	−3.9	0.0575*	0.5
Samples: D: A	8	.0000155	0.5	0.015	2.6	0.057	6.1	−0.0232	−0.2
Duplicates: S: D: A	16	.0000477	1.4	0.032	5.7	0.081	8.6	0.1222	1.2
Total	31	.0032688	100.0	0.566	100.0	0.932	100.0	10.2368	100.0

* Statistically significant ($P < 0.05$).
** Statistically significant ($P < 0.01$).

TABLE 3

Estimates of precision of BSP clearance measures as determined by two photometric methods for plasma blank correction

Item	Photometric method			
	Original		Improved	
	Calves	Cows	Calves	Cows
Number of animals	10	11	4	4
Mean BSP fractional clearance	0.1348	0.1812	0.1678	0.1897
S.E. of a single determination	0.0283	0.0285	0.0199	0.0120
C.V. of a single determination *(%)*	20.99	15.71	11.89	6.33
Mean plasma volume *(% B.W.)*	8.666	5.107	8.189	3.860
S.E. of a single determination	2.305	1.003	1.386	0.207
C.V. of a single determination *(%)*	26.60	19.64	16.92	5.36
Mean blood volume *(% B.W.)*	15.10	7.64	14.80	5.62
S.E. of a single determination	4.04	1.38	2.71	0.32
C.V. of a single determination *(%)*	26.74	17.99	18.34	5.66
Mean BSP volume clearance milliliter per minute per kilogram B.W.	11.17	9.21	13.74	7.52
S.E. of a single determination	1.62	1.48	1.24	0.40
C.V. of a single determination *(%)*	14.49	16.04	9.05	5.27

DISCUSSION

From these various studies it is clear that the improved photometric technique for the determination of sulfobromophthalein in plasma largely eliminates the effect of any hemolysis on the measurement. Hemolysis of blood is frequently encountered in routine blood sampling procedures and seriously interferes with the measurement of this dye.

The necessity of developing a regression equation for predicting basic plasma blank O.D. from acidic plasma blank O.D. may constitute a severe limitation on the use of the method. However, it is felt that relatively simple graphic procedures based on only a few acid-base plasma blank comparisons of hemolyzed plasmas might suffice as a basis for obtaining basic plasma blank O.D. If no hemolyzed plasma samples were present in a trial, the more direct methods of BSP assay would be indicated.

REFERENCES

(1) Cornelius, C. E., and Wheat, J. D. Bromsulphalein Clearance in the Horse—A Quantitative Liver Function Test. Am. J. Vet. Research, 18: 369. 1957.

(2) Goodman, R. D. Bromsulphalein Clearance: A Quantitative Clinical Test of Liver Function. J. Lab. Clin. Med., 40: 531. 1952.

(3) Hawk, P. B., Oser, B. L., and Summerson, W. H. Practical Physiological Chemistry. 12th ed., p. 559. The Blakiston Company, New York. 1951.

(4) Lavers, G. D., Cole, W. H., Keeton, R. W., Gebhardt, M. C., and Dyniewicz, J. M. Bromsulphalein Clearance. J. Lab. Clin. Med., 34: 965. 1949.

(5) Mixner, J. P., and Robertson, W. G. Bromsulphalein Fractional Clearance in Dairy Cattle as a Criterion of Liver Function, and the Simultaneous Determination of Volumes of Plasma and Blood. J. Dairy Sci., 40: 914. 1957.

(6) Rosenthal, S. M., and White, E. C. Clinical Application of Bromsulphalein for Hepatic Function. J. Am. Med. Assoc., 84: 1112. 1925.

IMPROVED PHOTOMETRIC METHOD FOR ASSAYING PHENOLSULFONPHTHALEIN IN THE BLOOD OF CATTLE IN TESTS FOR RENAL FUNCTION[1]

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SUMMARY

The usual photometric procedures for the assay of phenolsulfonphthalein (PSP) in plasma containing free hemoglobin in renal function studies have been found to be inadequate. An improved method of photometric assay for PSP in plasma has been developed in which plasma blank optical densities (O.D.) of a series of blood samples containing no dye, but with varying degrees of induced hemolysis, were measured both in acidic and in basic media. Basic plasma blank O.D. were predicted from acidic plasma blank O.D. by means of derived regression equations. In the routine assay of PSP in plasma, O.D. readings were first made on the acidified plasmas. Excess base was then added and O.D. readings again made on the sample. When the predicted basic plasma blank O.D. were used to correct the O.D. of the dye-containing plasma samples (varying amounts of hematin), accurate estimates of dye content were obtained. In actual renal function tests in both calves and cows the improved photometric method was superior to the original procedure in the precision with which the measures were made.

The acid-base photometric procedure previously employed for the assay of phenolsulfonphthalein (PSP) in the blood plasma of cattle in tests for renal function (1, 2, 5) was found by experience to be deficient in two respects. First, although the turbidity normally exhibited by plasma or plasma diluted by water or saline can usually be cleared by the addition of dilute acid or base, in some instances this turbidity was not eliminated by such a procedure, resulting instead in a poor plasma blank. Secondly, if hemoglobin was present in the plasma sample, the acid hematin formed in the blank had a different optical density (O.D.) than the alkali hematin of the split plasma sample, again leading to poor blank correction.

Manning *et al.* (4) modified the procedure of Mixner and Anderson (5) for use with sheep, because of the turbidity problem. Plasma samples containing hemoglobin were not used; although this eliminated the problem of hemoglobin, it meant the loss of some potential data. With the need for acidic plasma blanks eliminated, basic plasma blanks were run on plasma of blood collected prior to the intravenous injection of the dye.

In our experience hemolysis is particularly prone to occur in blood samples collected from calves and sporadically in samples in general. To be able to salvage trials in which hemolysis of blood samples had occurred, a photometric adaptation was made. This modification was found to be useful in the assay of sulfobromophthalein (BSP) in blood plasma of cows (3) in a test for hepatic

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function. It involved the development of a regression equation, such that basic plasma blank O.D. values were predicted from the corresponding acidic plasma blank O.D. values made on the plasma samples containing dye. Thus, the O.D. of each dye-containing plasma was first read in an acidic medium, after which excess base was added and the O.D. again determined to measure dye intensity. From the acidic plasma O.D. reading, a corresponding basic plasma blank O.D. was predicted by the equation, such predicted values being used as a correction for the basic O.D. dye readings.

METHODS AND PROCEDURES

Photometric procedure. To eliminate the turbidity problem, the ratio of plasma to total acid diluent in the photometric procedure was changed from 1 in 2.5 to 1 in 5. The dilution in the amount of dye was adjusted by increasing the light path in the colorimeter tubes from 11 to 23 mm. Alternatively, to correct for dye dilution a greater amount of dye could be injected per animal.

The modified photometric procedure is as follows: Using a round colorimeter tube (23 mm. i.d.), to 3.0 ml. of plasma are added 9.0 ml. of water and 3.0 ml. of 0.5 *N* HCl, and these materials mixed thoroughly. The tube is allowed to stand for 10 min., to develop the acid hematin if present. The O.D. of the tube is then read against a water blank in a spectrophotometer at 560 mμ (a Beckman Model B Spectrophotometer equipped with an adjustable tube holder was used). Immediately after this reading is made, 1.5 ml. of 2.5 *N* NAOH is added to the tube and mixed by swirling. The O.D. is then read immediately in the spectrophotometer a second time at 560 mμ.

Development of the regression equation. The procedures for the development of the regression equation for predicting basic plasma blank O.D. from acidic plasma blank O.D. were identical to those used in the modification for measuring sulfobromophthalein in hemolyzed blood (3).

Test of the accuracy of the improved photometric method. The accuracy of the improved photometric method was tested in a manner identical to that used for sulfobromophthalein assay (3). Simulated PSP fractional clearance tests were devised, whereby either 0.018 or 0.009 mg. of PSP was added to tubes containing the four possible combinations of clear and hemolyzed plasmas, to simulate those levels of PSP generally encountered in the 30-min. and 60-min. plasma samples in an actual test of renal function.

A similar series of tubes was run, using the original method of plasma blank correction. The PSP fractional clearance values obtained by the two photometric methods of blank correction were compared with a calculated theoretical PSP fractional clearance value.

Repeatability and precision of PSP clearance measures. PSP renal function tests were run in four bull calves and four cows to determine certain aspects of repeatability and precision, in a manner similar to that used for sulfobromophthalein fractional clearance tests for hepatic function (3). The data from the above trials were compared with similar data previously collected by use of the original photometric method (1).

RESULTS

Development of the regression equation. A regression equation was calculated by the method of least squares to predict the O.D. of basic plasma blanks from their corresponding acidic plasma blanks. This regression equation calculated on a within plasma sample basis was based on a series of four plasma samples, each having a series of seven subsamples with varying degrees of hemolysis.

The regression equation developed was $Y = 1.123\ X - 0.0109$, in which Y equaled the O.D. of the basic plasma blanks and X equaled the O.D. of the acidic plasma blanks. In the example below, and using the photometric method as already described, the acidic plasma blank O.D. of 0.018 mg. of PSP was 0.168; whereas, the excess base PSP plasma O.D. was 0.561. Substituting the acidic plasma blank O.D. in the regression equation, a predicted basic plasma blank O.D. of 0.177 was obtained, giving an adjusted PSP plasma O.D. of 0.384.

	Plasma blank O.D.		PSP plasma O.D.	
PSP (mg.)	Acidic	Predicted basic	Excess base	Adjusted
0.018	0.168	0.177	0.561	0.384

Test of the accuracy of the new photometric method. Both the original (1) and the improved photometric methods were used to obtain simulated PSP fractional clearance values when PSP was added to the four combinations of clear and hemolyzed plasma (Table 1). The improved photometric technique

TABLE 1

Comparison of the accuracy of original and improved photometric methods as used to determine simulated PSP fractional clearance values (four plasma samples)

Photometric method	Characteristic	Simulated PSP fractional clearance values			
		Plasma tube hemolysis combinations[a]			
		A	B	C	D
Original (5)	PSP fractional clearance				
	Theoretical mean	0.0231	0.0231	0.0231	0.0231
	Observed mean	0.0233	−0.0117	0.0453	0.0213
	Deviations from theoretical[b]	0.0013	0.0419	0.0275	0.0023
	Deviations from theoretical as per cent of theoretical mean[c]	5.63	181.39	119.05	9.96
Improved	PSP fractional clearance				
	Theoretical mean	0.0231	0.0231	0.0231	0.0231
	Observed mean	0.0237	0.0214	0.0242	0.0226
	Deviations from theoretical[b]	0.0009	0.0021	0.0013	0.0012
	Deviations from theoretical as per cent of theoretical mean[c]	3.90	9.09	5.63	5.19

[a] A 1st plasma tube clear; 2nd plasma tube clear
B 1st plasma tube clear; 2nd plasma tube hemolyzed
C 1st plasma tube hemolyzed; 2nd plasma tube clear
D 1st plasma tube hemolyzed; 2nd plasma tube hemolyzed.

[b] Calculated as $\sqrt{\frac{\Sigma\ (X_o - X_t)^2}{N-1}}$ where X_o and X_t equal the observed and theoretical mean PSP fractional clearance values, respectively. This is equivalent to a standard error of a single determination.

[c] This is equivalent to a coefficient of variation of a single determination.

was superior in all plasma combinations, as judged by the deviations of the observed mean PSP fractional clearances from the theoretical mean PSP fractional clearances as per cent of the theoretical mean PSP fractional clearances (Table 1).

Repeatability and precision of PSP clearance measures. The data for calves and cows were analyzed separately, since there were marked and significant differences between these classes of animals with respect to PSP clearance measures. The animal variances (repeatability estimates) for the various PSP clearance measures were low and may not be too representative of animal variance in larger groups of animals (Table 2). The standard error and coeffi-

TABLE 2

Estimated variances and variance percentages for PSP clearance measures using the improved photometric procedure

Source of variance	Degrees of freedom	PSP fractional clearance		PSP volume of distribution as per cent of body weight		PSP volume clearance as milliliters per minute per kilogram of body weight	
		Variance	Variance per cent	Variance	Variance per cent	Variance	Variance per cent
Bull calves							
Animal	3	.00000319	33.9	49.65	62.2	2.265	61.8
Days: A	4	.00000403	42.8	26.33	33.0	1.374	37.5
Samples: D: A	8	.00000112	11.9	1.71	2.1	0.016	0.4
Duplicates: S: D: A	16	.00000107	11.4	2.16	2.7	0.011	0.3
Total	31	.00000941	100.0	79.85	100.0	3.666	100.0
Cows							
Animal	3	.00000097	15.7	0.437	4.8	−0.0081	−1.1
Days: A	4	.00000329	53.4	7.564	82.8	0.7077	95.2
Samples: D: A	8	.00000007	1.3	−0.053	−0.6	0.0180	2.4
Duplicates: S: D: A	16	.00000182	29.6	1.191	13.0	0.0262	3.5
Total	31	.00000615	100.0	9.139	100.0	0.7438	100.0

TABLE 3

Estimates of precision of PSP clearance measures as determined by two photometric methods for plasma blank correction

Item	Photometric method			
	Original (1)		Improved	
	Calves	Cows	Calves	Cows
Number of animals	20	22	4	4
Mean PSP fractional clearance	0.0256	0.0435	0.0238	0.0370
S.E. of a single determination	0.0034	0.0055	0.0025	0.0023
C.V. of a single determination *(%)*	13.1	12.7	10.5	6.2
Mean PSP volume of distribution as per cent of body weight	31.8	18.2	32.1	21.8
S.E. of a single determination	7.6	4.5	5.5	3.0
C.V. of a single determination *(%)*	23.9	24.7	17.1	13.5
Mean PSP volume clearance, as milliliters per minute per kilogram body weight	7.89	7.77	7.58	8.02
S.E. of a single determination	1.90	1.25	1.18	0.87
C.V. of a single determination *(%)*	24.1	16.2	15.6	10.8

cients of variation of single determinations are probably the best measures of the precision of PSP clearance measures. These are presented in Table 3, in which these measures are calculated as the square roots of the sum of variances for days in animal, samples in days in animals, and duplicates in samples in days in animals as taken from Table 2. The precision of making PSP clearance measures by the improved photometric technique was greater in all instances than the precision of the original photometric method (1).

DISCUSSION

For the routine measurement of PSP in the plasma of cattle containing no free hemoglobin, a procedure similar to that reported by Manning *et al.* (4) would be preferable, in which a basic plasma blank containing no dye is used as a correction for the PSP-containing plasma. If any of the plasma samples contain free hemoglobin, this procedure is not adequate. The photometric method reported here largely corrects for the presence of hemoglobin in plasma and, thus, is a more versatile procedure. In lieu of regression analysis, as used in this study to develop the basis for predicting the basic plasma blanks of plasmas containing hemoglobin, simpler graphic procedures could be used to advantage.

REFERENCES

(1) Anderson, R. R., and Mixner, J. P. Normal Standards, Repeatability, and Precision of Phenolsulfonphthalein Clearance Measures for Renal Function in Dairy Cattle. J. Dairy Sci., 42: 545. 1959.

(2) Anderson, R. R., and Mixner, J. P. Effect of Synthalin A on Renal Function in Dairy Bull Calves as Measured by Phenolsulfonphthalein Clearance. J. Dairy Sci., 42: 1387. 1959.

(3) Anderson, R. R., and Mixner, J. P. Correction for Hemolysis in a Photometric Method for Assaying Sulfobromophthalein in the Blood of Cattle in Tests for Hepatic Function. J. Dairy Sci., 43: 1465. 1960.

(4) Manning, J. P., Reber, E. F., Malhotra, O. P., Beamer, P. D., Boley, L. E., and Norton, H. W. Phenolsulfonphthalein Clearance as a Measure of Renal Function in Ewes. Am. J. Vet. Research, 20: 858. 1959.

(5) Mixner, J. P., and Anderson, R. R. Phenolsulfonphthalein Fractional Clearance in Dairy Cattle as a Measure of Renal Function. J. Dairy Sci., 41: 306. 1958.

INULIN RENAL CLEARANCE IN DAIRY CATTLE [1,2]

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SUMMARY

A practical method for determining inulin renal clearance (the glomerular filtration rate of the kidneys) and the volume of the extracellular fluids of cattle is described, based on the measurement of the kinetics of inulin distribution and disposal after intravenous infusion. For these purposes the blood level of inulin was determined at varying time intervals up to 2 hr. after infusion.

Inulin had reached equilibrium with the extracellular fluids of the body in approximately 45 min. (half-time of about 6 min.). The volume of the distribution of inulin was calculated to be 29.3 and 15.3% of body weight in a calf and cow, respectively. The inulin fractional clearance rate in a calf and cow were 0.0089 and 0.0168 per minute, while the corresponding inulin volume clearances were 2.61 and 2.57 ml. per minute per kilogram of body weight, respectively.

From a variety of comparative studies it has been concluded that the renal clearance of the polysaccharide, inulin, is a measure of the renal glomerular filtration rate in vertebrates (6), including the cow (3). In addition, it has been found that inulin is well suited for the measurement of the fluid compartment of the body, consisting of the plasma and extracellular fluids (6).

Poulsen (3) reported that the mean inulin clearance of ten cows was 1.84 ml. per minute per kilogram of body weight. Inulin clearance was found to be independent of diuresis or plasma concentration of inulin. Sellers *et al.* (5) reported that inulin clearances in calves 1-2 wk. of age, calves 6-10 wk. of age, and heifers 1½-2½ yr. of age were 112, 169, and 151 ml. per minute per square meter of body surface, respectively. According to Smith (6), the inulin space of the body is about 16% of body weight.

In studies on renal function in the cow (1, 2), phenolsulfonphthalein (PSP) was shown to be eliminated from the blood at a rate characterized by an exponential or first-order reaction, leading to the calculation of PSP fractional clearance and clearance measures based only on plasma concentration of PSP, urine analyses not being required.

This study was initiated to determine if the renal clearance of inulin might be measured by similar techniques. Of particular interest were the time rela-

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[2] This study was part of a Northeast Regional Research Project (NE-41, Endocrine Factors Affecting Reproduction and Lactation in Dairy Cattle), a cooperative study involving agricultural experiment stations in the Northeastern Region and supported in part by regional funds of the United States Department of Agriculture.

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tionships necessary for the determination of inulin clearance, using blood measures of inulin after intravenous infusion.

METHODS AND PROCEDURES

Ten and 40 g. of inulin were injected rapidly into the jugular veins of Holstein Bull Calf 973[8] and Guernsey Cow 981, respectively. Small blood samples were collected before infusion, at approximately 4-min. intervals during the first half hour, and at 15-min. intervals during the next 1.5 hr. after infusion. The exact times of inulin infusion and collection of blood samples were noted accurately.

Inulin in plasma samples was assayed by the direct resorcinal method without alkali treatment, as described by Schreiner (4).

The plasma inulin time curves were analyzed by statistical methods previously described for renal studies, using phenolsulfonphthalein (1, 2).

RESULTS AND DISCUSSION

It was determined that disappearance of inulin from the blood plasma of the two animals could be characterized by the general formula of a compound exponential regression, inulin, milligrams per milliliter plasma $= a_1e^{-b_1t} + a_2e^{-b_2t}$ in which

a_1 = concentration of inulin (milligram per milliliter) at zero time by extrapolation of the more rapid initial distribution curve.
$-b_1$ = slope (rate constant) of distribution curve or phase.
a_2 = concentration of inulin (milligram per milliliter) at zero time by extrapolation of the slower disposal curve.
$-b_2$ = slope (rate constant) of the slower disposal curve.
e = base of natural logarithms.
t = time in minutes from dye injection.

The first part of this equation ($a_1e^{-b_1t}$) represents the initial rapid decline in inulin level due to the distribution of inulin from the plasma into other extracellular fluids. The second part of the equation ($a_2e^{-b_2t}$) represents the elimination or disposal of inulin from its space or volume of distribution by glomerular filtration after distribution has been completed.

The volume of the extracellular fluids of the body (volume of distribution of inulin) may be calculated from the formula

$$\text{V.D., milliliter} = \frac{\text{milligram of inulin injected}}{a_2}$$

The biological half-time ($t_{1/2}$) for either the inulin distribution or disposal phase is calculated by dividing the natural logarithm of two (0.693) by b_1 or b_2, respectively.

The various constants of these equations for the two trials are presented in Table 1.

The half-times for the distribution of inulin into the extracellular fluids of the body were 5.42 and 5.88 min. Accordingly, seven half-times, when dis-

TABLE 1

Kinetic characteristics of the distribution and disposal of inulin in dairy cattle

Characteristic	Calf H973 [a]	Cow G981
Body weight *(kg.)*	44.3	466
Dosage inulin *(g.)*	10	40
Distribution curve		
Degrees of freedom (N−2)	4	5
r[a]	−0.98	−0.99
a_1, milligram inulin per milliliter	1.236	0.833
$-b_1$ *(per minute)*	0.1280	0.1178
$t_{1/2}$ *(min.)*	5.42	5.88
Disposal curve		
Degrees of freedom (N−2)	5	5
r[a]	−0.98	−0.99
a_2, milligram inulin per milliliter	0.771	0.3386
$-b_2$, per minute (fractional clearance)	0.0089	0.0168
$t_{1/2}$ *(min.)*	77.8	41.3
Inulin volume of distribution[b]		
Total *(ml.)*	12,963	71,431
Per cent of body weight	29.3	15.3
Inulin volume clearance		
Milligrams per minute	115.5	1197.8
Milligrams per minute per kilogram body weight	2.61	2.57
Milligrams per minute per meter[2] body surface	90.9	256

[a] The coefficient of correlation between time in minutes after dye injection and log. of inulin concentrations of the first-order regressions.

[b] Measure of the volume of the extracellular fluids of the body, including plasma and lymph.

tribution would be approximately 99% complete, would be approximately 45 min. The half-times for the disposal of inulin were 77.8 and 41.3 min. From these considerations it is recommended that if only two blood samples are to be used to characterize inulin fractional clearance (b_2), they should be collected at approximately 45 and 105 min. after the infusion of inulin.

The volume of distribution of inulin (29.3 and 15.3% of body weight in calf and cow, respectively) is approximately what would be expected as the volume of the extracellular fluids of the body. These volumes are quite similar to those reported for phenolsulfonphthalein in calves and cows (1, 2).

The inulin volume clearances (2.61 and 2.57 ml. per minute per kilogram of body weight in calf and cow, respectively) are much lower than those reported for phenolsulfonphthalein (7.48 and 8.00 ml. per minute per kilogram of body weight in calves and cows, respectively), using the same general techniques. The differences in inulin and phenolsulfonphthalein volume clearances represent the differences in their mode of elimination, the inulin being removed by glomerular filtration only, while the phenolsulfonphthalein is removed both by glomerular filtration and proximal tubule secretion.

Both inulin fractional clearance ($-b_2$) and volume clearance are considered to be dynamic measures of renal glomerular function. They are easily measured without need of collecting urine, although the inulin volume clearance corresponds directly to clearance values obtained by usual methods of plasma and urine analysis (6).

These preliminary trials in cattle define the general time relationships of inulin distribution and disposal inherent in a practical test of renal function

based on glomerular filtration rates as determined only from plasma analyses of inulin concentrations.

REFERENCES

(1) Anderson, R. R., and Mixner, J. P. Normal Standards, Repeatability, and Precision of Phenolsulfonphthalein Clearance Measures for Renal Function in Dairy Cattle. J. Dairy Sci., 42: 545. 1959.

(2) Mixner, J. P., and Anderson, R. R. Phenolsulfonphthalein Fractional Clearance in Dairy Cattle as a Measure of Renal Function. J. Dairy Sci., 41: 306. 1958.

(3) Poulsen, E. Renal Clearance in the Cow. Royal Veterinary and Agricultural College Yearbook. Copenhagen, Denmark. 1957.

(4) Schreiner, G. E. Determination of Inulin by Means of Resorcinol. Proc. Soc. Exptl. Biol. Med., 74: 117. 1950.

(5) Sellers, A. F., Pritchard, W. R., Weber, A. F., and Sautter, J. H. Renal Function Studies in Normal Dairy Cattle and Those with Post-parturient Albuminuria. Am. J. Vet. Research, 19: 580. 1958.

(6) Smith, H. W. Principles of Renal Physiology. Rev. ed. Oxford University Press, New York. 1956.

EFFICIENCY OF ABSORPTION OF THYROXINE IN VARIOUS FORMS FROM THE GASTROINTESTINAL TRACTS OF LACTATING COWS[1,2]

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SUMMARY

The efficiencies of absorption of orally administered crystalline L-thyroxine, thyroprotein, and thyroglobulin from the gastrointestinal tract of lactating cows were estimated by comparing the relative increase of plasma protein-bound iodine (PBI) from oral administration of these materials, with the corresponding PBI increases due to intravenous administration of L-thyroxine.

In seven lactating cows the mean efficiency of absorption of thyroxine from thyroprotein (1.06% L-thyroxine) was 12.3%. Duplicate trials of absorption efficiency were performed in six other cows, the mean absorption efficiency being 13.9%; animal effects were highly significant statistically.

Five lactating cows were used to determine the absorption efficiency of crystalline L-thyroxine and thyroxine in porcine thyroglobulin concentrate (0.37% L-thyroxine). Again, highly significant differences were found among animals. The mean absorption efficiencies of the crystalline L-thyroxine and the thyroxine from thyroglobulin were 11.6 and 15.9%, respectively.

These results suggest that one of the important causes of animal variation in their lactational (or other) responses to orally administered thyroidal materials may be the efficiency with which the materials are absorbed from the digestive tract.

Relatively few data are available regarding the comparative effectiveness of orally and parenterally administered thyroidal materials in ruminants. In sheep, Turner and Reineke (21) found that thyroprotein administered orally was only 5% as effective in causing body weight reductions as that administered subcutaneously. In the same type of experiments another thyroprotein preparation was only 3% as effective orally as subcutaneously. Turner and Reineke (21) also reported that the sodium salts of D,L-thyroxine were about 12.5% as effective orally as subcutaneously, based upon the amounts of materials required to cause body weight losses in sheep.

Monroe and Turner (15) fed thyroprotein to goats and calculated the efficiency of absorption by comparing the crystalline D,L-thyroxine equivalents of the feed and feces. The estimated absorption efficiency of 78 to 88% was undoubtedly much too high, since by this technique it was not possible to distinguish between the amount of destruction of thyroxine in the digestive tract and the amount of absorption of thyroxine from the tract.

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[2] This study was part of a Northeast Regional Research Project (NE-30, The Relation of Certain Physiological Factors to the Productive Capacity of Dairy Cattle), a cooperative study involving agricultural experiment stations in the Northeastern Region and supported in part by regional funds of the United States Department of Agriculture.

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More recently, Wagner (22) compared the relative efficiency of injected L-thyroxine with orally administered thyroprotein (1% L-thyroxine equivalent) in blocking the release of I^{131} from the thyroid gland of ewes. Two-tenths of a milligram of L-thyroxine injected was equivalent to 1.8 mg. L-thyroxine (in thyroprotein) fed, for an efficiency of absorption of 11.1%.

In dairy cows there seem to be no comparisons of the relative effectiveness of orally and parenterally administered thyroxine. Bailey *et al.* (1) showed that oral doses of 50 to 150 mg. of L-thyroxine daily caused marked lactational responses in cows. Based upon lactation responses, it is generally considered that daily doses of 15 g. of thyroprotein (1% of L-thyroxine) or 150 mg. of L-thyroxine administered orally are equivalent to 15 mg. of injected L-thyroxine, for an oral efficiency of approximately 10%.

The purpose of this study was to assess the relative efficiencies of absorption of oral L-thyroxine administered as thyroprotein, crystalline L-thyroxine, or thyroglobulin. This was done by comparing the increase in the plasma protein-bound iodine (PBI) caused by orally administered thyroxine materials to the PBI increases caused by intravenous L-thyroxine as a reference standard.

Thyroxine is the major form of PBI in normal plasma (4, 16, 20) and both exogenous and endogenous thyroxine in plasma are protein-bound in the same way and have identical protein-linkage (6).

To what extent PBI represents blood thyroxine in cattle may be judged from a number of studies. Long *et al.* (11) demonstrated that PBI is increased by thyroprotein feeding. Mixner and Lennon (13, 14) have shown the quantitative aspects of the effects of single administrations of exogenous thyroprotein, L-thyroxine, and L-triiodothyronine on PBI in cattle. Long *et al.* (12) found that a daily oral supplement of 22 mg. of KI had no effect on PBI, and that larger doses caused an increase. King and Lee (7) and Lee *et al.* (8) have shown that the feeding of a trace-mineralized salt containing 3,5-diiodosalicylic acid and cuprous iodide caused marked increases in the level of plasma PBI. Lewis (10) reported that the PBI level in an I^{131}-radiation-thyroidectomized bull dropped to 0.8 μg. % in 33 days from a control level of 6.7 μg. %. Reece and Man (17) reported that only 57.2% of bovine sera PBI was butanol extractable (presumably thyroxine iodine).

Iodinated casein (a thyroprotein) has been shown by Friedberg and Reineke (5) to contain iodide, monoiodotyrosine, diiodotyrosine, thyroxine, and possibly triiodothyronine. Similarly, the iodine of thyroglobulin consists of a few per cent as monoiodotyrosine, about 60% as diiodotyrosine, about 30% as thyroxine, with traces of triiodothyronine and other iodine compounds (23). The extent to which these various substances would be absorbed from the digestive tract of cattle and become plasma protein-bound is not known with any degree of certainty.

From these various reports it is evident that thyroxine as well as nonthyroxine iodine may cause increases in plasma PBI. It seemed feasible, however, to investigate the use of increases in PBI levels in cattle as an index of absorption efficiency of orally administered thyroidal materials.

MATERIALS AND METHODS

The L-thyroxine for intravenous injections in all experiments was in the form of sodium L-thyroxine pentahydrate.[4] Dosages are reported as L-thyroxine equivalent and corrected for purity.

The thyroprotein[5] (iodinated casein) used in these trials was assayed chemically by Cerophyl Laboratories to be 1.06% L-thyroxine equivalent, by a method claimed to give results similar to those obtained by a radioisotope dilution technique (18). Its content of diiodotyrosine was about 6%.

L-thyroxine[6] for oral administration was in the form of sodium L-thyroxine pentahydrate. Lactose was used as a carrier (4.0 g. thyroxine per 100 g. lactose). Dosages are reported as L-thyroxine equivalent, corrected for purity.

The porcine thyroglobulin concentrate[7] was assayed by Warner-Lambert Research Institute to be 0.83% total iodine and 0.24% Blau or thyroxine iodine, which would be the equivalent of 0.37% L-thyroxine.

Plasma PBI was assayed by the method of Brown *et al.* (3), as modified by Lennon and Mixner (9).

All cows used in these studies were lactating.

EXPERIMENTAL PROCEDURES

A previous study (13) has shown that exogenous thyroxine is eliminated from the plasma in a manner characterized by a first-order or exponential regression. Using this scheme, the theoretical level of thyroxine in its volume of distribution may be calculated as of zero time, after distribution is completed but before any is eliminated (a_2 value or Y-axis intercept where $Y =$ PBI values and $X =$ time after thyroxine administration).

From the above considerations it was proposed to compare the PBI increase per milligram thyroxine fed to the PBI increase per milligram of thyroxine injected intravenously to derive an efficiency of absorption for thyroxine. The accuracy of such comparisons will depend in part on the accuracy of determinations of thyroxine in orally administered materials and also in part on the specificity with which thyroxine alone in orally administered materials will cause increases in PBI as a measure of thyroxine level.

Preliminary studies. As a basis for routine study, three cows were given orally by capsule 75 g. of thyroprotein, 100 g. of thyroglobulin concentrate, and 300 mg. of L-thyroxine, respectively, to determine the time relationships with respect to the absorption of these materials as reflected in PBI increases.

A single cow (H804), in successive trials, was given 25.2, 50.5, and 75.7 mg. of L-thyroxine intravenously, and 25, 50, and 75 g. of thyroprotein by capsule

[4] Supplied through the courtesy of Dr. H. L. Saunders, Smith, Kline, and French Laboratories, Philadelphia.

[5] Thyroprotein was generously supplied by Mr. Loren V. Burns of the Cerophyl Laboratories, Inc., Kansas City.

[6, 7] We are indebted to Dr. Robert L. Kroc, Warner-Lambert Research Institute, Morris Plains, New Jersey, for the supply of L-thyroxine for oral administration (Preparation No. W1563-4) and the thyroglobulin concentrate (W1712, Lot No. 10477).

orally, to determine the effect of the dosage of these materials on the PBI increase per milligram of thyroxine administered by the two methods, respectively. Following oral administration of thyroprotein in capsular form, blood samples were collected at 36 and 72 hr. afterwards. Following intravenous L-thyroxine administration, blood samples were collected at 24 and 60 hr.

Thyroprotein study. Trial 1. Seven cows in successive periods were each given single oral doses by capsule of 25 g. of thyroprotein (265 mg. of L-thyroxine equivalent) and single intravenous doses of 25.2 mg. of L-thyroxine. Blood samples were collected as outlined previously.

Trial 2. To assess the differences among and within cows associated with the estimates of absorption efficiency, duplicate procedures were performed in each cow at 1-mo. intervals, such that each cow received in each trial a single oral dose by capsule of 25 g. of thyroprotein and 25.2 mg. of L-thyroxine intravenously. Blood samples were collected as previously outlined.

Oral thyroxine and thyroglobulin trial. Five cows were used to study the efficiency of absorption of orally administered L-thyroxine and thyroglobulin concentrate. At 2-wk. intervals, and in a somewhat random order, each cow received successively 25.2 mg. of L-thyroxine intravenously, 300 mg. or 450 mg. of L-thyroxine orally by capsule, and 75 or 100 g. of thyroglobulin orally by capsule. Blood samples were collected as outlined previously. Since, from this scheme, the efficiency of absorption of both L-thyroxine and thyroglobulin was determined within each animal, an error term (animal × thyroxine source) would allow the assessment of animal differences with respect to efficiency of absorption.

RESULTS

Preliminary studies. Figure 1 presents the time relationships with respect to the absorption of thyroxine from the gastrointestinal tract in the form of orally administered L-thyroxine, thyroprotein, and thyroglobulin, as reflected in PBI increases. PBI was plotted on a logarithmic scale (Y-axis), whereas time was plotted on a linear scale (X-axis). From this graph and other preliminary work it was concluded that blood samples taken 36 hr. and afterwards from the time of oral administration of the thyroidal materials could be used to define the semilogarithmic regression.

The effects of varying dosages of either intravenous L-thyroxine or oral thyroprotein on PBI increases per unit of thyroidal material administered are shown in Table 1. The efficiency of absorption of the highest dose of thyroprotein perhaps was depressed somewhat. Apparently, this was not a reflection of the thyroxine-binding capacity of the blood, since PBI increases per unit of thyroxine injected intravenously were approximately the same for all dose levels. In subsequent trials, the lower doses of intravenous L-thyroxine and oral thyroprotein were used, since these gave adequate PBI responses for analytical purposes.

Thyroprotein study. Trial 1. The efficiency of absorption of thyroxine in thyroprotein from the gastrointestinal tract was estimated in seven cows (Table

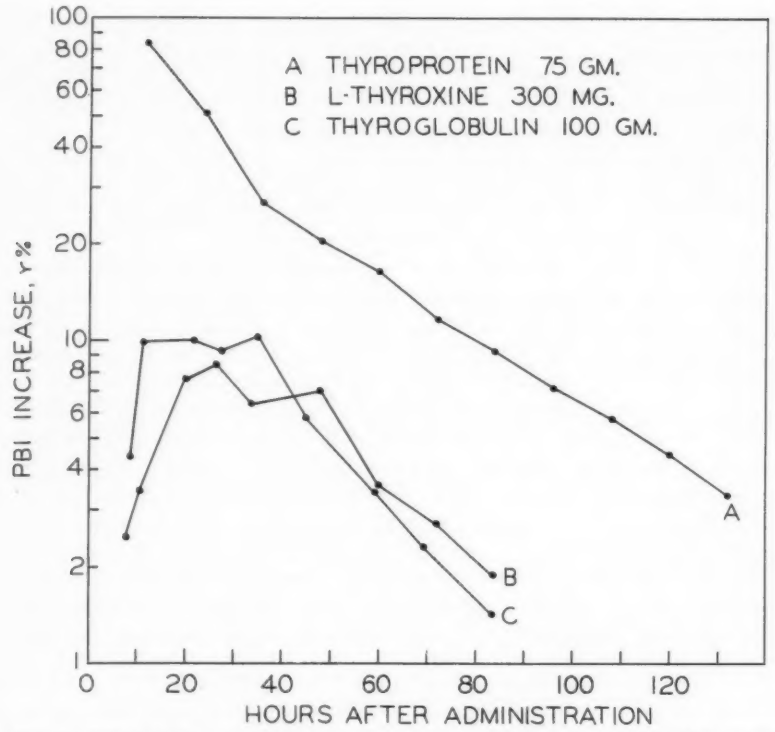

FIG. 1. Effect of time on PBI increase after oral administration of thyroidal materials. Note that at about 36 hr. after administration, absorption and distribution of thyroxine is largely completed and the elimination phase is progressing in a simple exponential manner.

2). The mean absorption efficiency was 12.3%, with a range from 8.3 to 17.3%. From this trial it could not be determined whether significant differences existed among cows with respect to absorption efficiency, although the range was large.

Trial 2. Duplicate estimates of the efficiencies of absorption of L-thyroxine from orally administered thyroprotein are presented for each of six cows (Table 3). The mean absorption efficiency was 13.9%, with a range from 7.7 to 22.4%. An analysis of variance of the absorption efficiency data (Table 4) showed that 60% of the total variance was attributable to cows, this figure being a short-time (1 mo.) repeatability estimate. The standard error of a single determination was 2.84%, with a coefficient of variation of 20.43%.

Oral thyroxine and thyroglobulin. The mean efficiencies of absorption of oral L-thyroxine and L-thyroxine in thyroglobulin were 11.62 and 15.86%, respectively (Table 5). The range for L-thyroxine was 4.45 to 18.38%, whereas the corresponding range for thyroglobulin was 6.52 to 25.62%. An analysis of variance of the efficiency of absorption data (Table 6) showed that there

TABLE 1

Effect of various dosages of intravenous L-thyroxine and oral thyroprotein on PBI increases in a single cow (H804)

Comparison	I.V. L-thyroxine		Oral thyroprotein			Efficiency of absorption of thyroxine in thyroprotein[a]
			Dosage			
	Dosage	PBI increase per milligram thyroxine	Actual	L-thyroxine equivalent	PBI increase per milligram thyroxine	
	(mg.)	*(μg. %)*	*(g.)*	*(mg.)*	*(μg. %)*	*(%)*
1	25.2	0.833	25	265	0.117	14.0
2	50.5	0.800	50	530	0.129	16.1
3	75.7	0.916	75	795	0.097	10.6
Means		0.850			0.114	13.6

[a] PBI increase per milligram thyroxine fed divided by PBI increase per milligram thyroxine injected, times 100.

TABLE 2

Efficiency of absorption of L-thyroxine from orally administered thyroprotein

Cow No.	I.V. L-thyroxine		Oral thyroprotein			Efficiency of absorption of thyroxine in thyroprotein[a]
			Dosage			
	Dosage	PBI increase per milligram thyroxine	Actual	L-thyroxine equivalent	PBI increase per milligram thyroxine	
	(mg.)	*(μg. %)*	*(g.)*	*(mg.)*	*(μg. %)*	*(%)*
H671	25.2	0.849	25	265	0.126	14.8
H704	25.2	1.207	25	265	0.100	8.3
H858	25.2	1.066	25	265	0.094	8.8
H711	25.2	0.762	25	265	0.104	13.7
H791	25.2	1.238	25	265	0.114	9.2
H856	25.2	0.865	25	265	0.150	17.3
H804	25.2	0.833	25	265	0.117	14.0
Means		0.974			0.115	12.3

[a] See footnote Table 1.

were highly significant differences among cows ($P < 0.01$) and significant differences ($P < 0.05$) between the thyroxine sources with respect to absorption efficiencies. A variance study (Table 6) showed that 81.6% of the total variance was attributable to cows, 13.0% due to thyroxine source, and 5.38% due to error variance (cow × thyroxine source). If thyroxine source variance is discounted, then 93.8% of total variance was due to cows (repeatability estimate) and 6.2% was due to error. The standard error of a single determination was 1.86% and the coefficient of variation was 13.5%.

DISCUSSION

The over-all biological effectiveness of an orally administered thyroidal material is the sum or product of its efficiency of absorption from the digestive tract and the physiological response of the animal to the quantity of materials

TABLE 3

Efficiency of absorption of L-thyroxine from orally administered thyroprotein as determined in duplicate in six cows

Cow No.	Trial No.	I.V. L-thyroxine: Dosage	I.V. L-thyroxine: PBI increase per milligram thyroxine	Oral thyroprotein: Dosage: Actual	Oral thyroprotein: Dosage: L-thyroxine equivalent	Oral thyroprotein: PBI increase per milligram thyroxine	Efficiency of absorption of thyroxine in thyroprotein[a]
		(mg.)	*(μg. %)*	*(g.)*	*(mg.)*	*(μg. %)*	*(%)*
H934	1	25.2	1.144	25	265	0.221	19.3
	2	25.2	1.759	25	265	0.217	12.4
G956	1	25.2	1.617	25	265	0.257	15.9
	2	25.2	1.719	25	265	0.251	14.6
H866	1	25.2	1.138	25	265	0.255	22.4
	2	25.2	1.414	25	265	0.241	17.1
H717	1	25.2	0.829	25	265	0.071	8.6
	2	25.2	0.893	25	265	0.069	7.7
H669	1	25.2	0.956	25	265	0.089	9.4
	2	25.2	0.696	25	265	0.091	13.1
G199	1	25.2	0.944	25	265	0.115	12.2
	2	25.2	0.777	25	265	0.110	14.1
Means			1.157			0.166	13.9

[a] See footnote Table 1.

TABLE 4

Analysis of variance and estimated variances of thyroprotein absorption efficiency data from duplicate estimates in each of six cows

Variance source	Degrees of freedom	Mean squares	Estimated variances: V.	Estimated variances: V. %
Among cows	5	32.15**	12.05	60.0
Between estimates within cows	6	8.05	8.05	40.0
Total	11		20.10	100.0

** Indicates statistical significance, $P < 0.01$.

TABLE 5

Efficiency of absorption of orally administered L-thyroxine and L-thyroxine in thyroglobulin

Cow No.	I.V. L-thyroxine: Dosage	I.V. L-thyroxine: PBI increase per milligram thyroxine	Oral L-thyroxine: Dosage	Oral L-thyroxine: PBI increase per milligram thyroxine	Oral thyroglobulin: Dosage: Actual	Oral thyroglobulin: Dosage: L-thyroxine equivalent	Oral thyroglobulin: PBI increase per milligram thyroxine	Efficiency of absorption: L-thyroxine	Efficiency of absorption: L-thyroxine in thyroglobulin
	(mg.)	*(μg. %)*	*(mg.)*	*(μg. %)*	*(g.)*	*(mg.)*	*(μg. %)*	*(%)*	*(%)*
H866	25.2	0.993	244.8	0.073	100	367.5	0.085	7.3	8.5
G950	25.2	1.255	244.8	0.056	75	275.6	0.082	4.5	6.5
H906	25.2	0.959	367.2	0.176	100	367.5	0.218	18.4	22.8
H908	25.2	0.941	367.2	0.093	100	367.5	0.149	9.8	15.9
H934	25.2	0.645	367.2	0.117	100	367.5	0.165	18.1	25.6
Means								11.6	15.9

TABLE 6

Analysis of variance and estimated variances of L-thyroxine and thyroglobulin absorption efficiency data

Source of variance	Degrees of freedom	Mean squares	Estimated variances		
			V.	V. %	V. %
Among cows (C)	4	107.85**	52.21	81.6	93.8
Thyroxine source (T)	1	45.07*	8.33	13.0	.....
C × T	4	3.44	3.44	5.4	6.2
Total	9		63.98	100.0	100.0

* and ** indicate statistical significance, $P < 0.05$ and $P < 0.01$, respectively.

actually absorbed. Thus, over-all biological effectiveness of orally administered thyroidal materials was measured in previous studies (21, 22). In contrast, it was the object of the present experiments to measure only the efficiency of absorption of the materials.

The estimates of the efficiency of absorption of thyroxine in thyroidal materials from the gastrointestinal tract as determined in these studies were quite similar to the over-all biological effectiveness of orally administered materials as found in sheep (21, 22).

It is interesting to note that the efficiencies of absorption of the different thyroidal materials, as measured by relative increases in plasma protein-bound iodine, were quite similar, ranging from 11.6 to 15.9%. These results tend to support the validity of the method as a measure of the efficiency of gastrointestinal absorption of thyroidal materials.

The marked variability among animals with respect to their lactational response to orally administered thyroidal materials is well known (2). Thomas *et al.* (19) found that the lactational response of cows appeared to be more limited by the individuality of the cow than by differences obtained by daily feeding of 1.0 or 1.5 g. of thyroprotein per 100 lb. of body weight. The extent of absorption of thyroactive materials from the gastrointestinal tract as a cause of animal differences may depend on such factors as the form in which thyroxine is fed, its solubility in water, and the extent of digestion or destruction. In addition, other physiological factors, such as age of the cow, stage of lactation, and potential producing ability may profoundly affect the lactational response of animals to given quantities of thyroidal materials. The nutritional status of the animal, managerial factors, and climate may all play their part.

There are no previous data that would indicate whether or not significant differences might exist among animals with respect to their ability to absorb orally administered thyroidal materials from their digestive tracts, as a possible cause of animal response differences. The present experiments indicate that there are large among-animal differences in the efficiency of absorption of thyroprotein, crystalline L-thyroxine, and thyroglobulin from the gastrointestinal tract, as measured by the relative increases in plasma PBI of intravenously and orally administered material.

The validity of among-animal significant differences in the efficiency of absorption of thyroidal materials from the digestive tract is not dependent in

these experiments on accurate measurements of the amount of L-thyroxine in the reference materials. The actual levels of efficiency of absorption were, of course, related directly to the levels of thyroxine contained in the various preparations, and to the degree to which PBI responses were due to thyroxine alone.

If animal differences in the efficiency of absorption of thyroidal materials were as great as these experiments would indicate, then it would be rather impossible to obtain uniform herd responses in lactation to orally administered thyroidal materials, unless maximum doses of thyroidal materials were given.

REFERENCES

(1) Bailey, G. L., Bartlett, S., and Folley, S. J. Use of L-Thyroxine by Mouth for Stimulating Milk Secretion in Lactating Cows. Nature, 163: 800. 1949.

(2) Blaxter, K. L., Reineke, E. P., Crampton, E. W., and Petersen, W. E. The Role of Thyroidal Materials and of Synthetic Goitrogens in Animal Production and an Appraisal of Their Practical Use. J. Animal Sci., 8: 307. 1949.

(3) Brown, H., Reingold, A. M., and Samson, M. The Determination of Protein-Bound Iodine by Dry Ashing. J. Clin. Endocrinol. and Metabolism, 13: 444. 1953.

(4) Deiss, W. P., Albright, E. C., and Larson, F. C. Comparison of in Vitro Serum Protein Binding of Thyroxine and Triiodothyronine. Proc. Soc. Exptl. Biol. Med., 84: 513. 1953.

(5) Friedberg, W., and Reineke, E. P. Thyroxine-like Compounds in Iodinated Protein and Thyroid Gland. Federation Proc., 11: March, 1952.

(6) Gordon, A. H., Gross, J., O'Connor, D., and Pitt-Rivers, R. Nature of the Circulating Thyroid Hormone-Plasma Protein Complex. Nature, 169: 19. 1952.

(7) King, W. A., and Lee, J., III. Source of Iodine in Salt Affects Protein-Bound Iodine Content of Bovine Blood Plasma. J. Dairy Sci., 42: 2003. 1959.

(8) Lee, J., III, King, W. A., and Conley, C. Protein-Bound Iodine in the Blood of Dairy Cattle Fed a Trace-Mineralized Salt. J. Dairy Sci., 43: 512. 1960.

(9) Lennon, H. D., Jr., and Mixner, J. P. Some Factors Affecting the Determination of Plasma Protein-Bound Iodine Using the Alkaline Fusion-Ceric Sulfate Method. J. Dairy Sci., 40: 351. 1957.

(10) Lewis, R. C. The Effect of Thyroidectomy and Iodine Supplementation of the Plasma PBI of a Jersey Bull. J. Dairy Sci., 39: 610. 1956.

(11) Long, J. F., Hibbs, J. W., and Gilmore, L. O. The Effect of Thyroprotein Feeding on the Blood Level of Inorganic Iodine, Protein-Bound Iodine, and Cholesterol in Dairy Cows. J. Dairy Sci., 36: 1049. 1953.

(12) Long, J. F., Hibbs, J. W., and Gilmore, L. O. The Effect of Feeding Different Amounts of Potassium Iodide on the Level of Protein-Bound Iodine in Cows. J. Dairy Sci., 37: 660. 1954.

(13) Mixner, J. P., and Lennon, H. D., Jr. Effects of Thyrotrophin, L-Thyroxine, and L-Triiodothyronine on Levels of Plasma Protein-Bound Iodine in Dairy Bull Calves. J. Dairy Sci., 41: 840. 1958.

(14) Mixner, J. P., and Lennon, H. D., Jr. Thyroxine Secretion Rates in Dairy Cattle as Calculated from Plasma Levels, Turnover Rates, and Volumes of Distribution of Thyroxine. Proc. XVth Intern. Dairy Congr., 1: 20. 1959.

(15) Monroe, R. A., and Turner, C. W. The Metabolism of Thyroxine. Missouri Agr. Expt. Sta., Research Bull. 446. 1949.

(16) Rall, J. E. Iodine Compounds in the Blood and Urine of Man. J. Clin. Endocrinol., 10: 996. 1950.

(17) Reece, R. P., and Man, E. B. Serum Precipitable and Butanol Extractable Iodine of Bovine Sera. Proc. Soc. Exptl. Biol. Med., 79: 208. 1952.

(18) REINEKE, E. P. The Thyroxine Content of Thyroactive Iodinated Proteins as Determined by a Radioactive Isotope Dilution Technique. J. Dairy Sci., 37: 1227. 1954.
(19) THOMAS, J. W., MOORE, L. A., AND SYKES, J. F. Some Effects of Feeding Thyroprotein to Dairy Cows During Their First Lactation. J. Dairy Sci., 32: 278. 1949.
(20) TONG, W., TAUROG, A., AND CHAIKOFF, I. L. Nature of Plasma Iodide Following Destruction of the Rat Thyroid with I^{131}. J. Biol Chem., 195: 407. 1952.
(21) TURNER, C. W., AND REINEKE, E. P. The Relation of the Route of Administration of Thyroxine, Thyroprotein, and Intermediate Products upon Their Utilization by Ruminants. Missouri Agr. Expt. Sta., Research Bull. 397. 1946.
(22) WAGNER, J. F. The Hormonal Control of Reproductive Activity in the Cycling and Anestrous Ewe. Ph.D. thesis, School for Advanced Graduate Studies, Michigan State University. 1959.
(23) WERNER, S. The Thyroid. p. 93. Hoebert and Harper, New York, New York. 1955.

HERITABILITIES OF SOME TYPE APPRAISAL TRAITS AND THEIR GENETIC AND PHENOTYPIC CORRELATIONS WITH PRODUCTION

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SUMMARY

Estimates of heritability for 27 type traits and milk and fat production and of genetic correlation of type traits with production were obtained by a within-herd-year analysis of 842 daughter-dam pairs of the Holstein-Friesian breed. Heritability estimates for fat and milk production were higher (.42 and .40) than most reported previously. Most of the type traits studied had heritability estimates not significantly different from zero. Those traits with relatively high estimates were: milking quality, depth of barrel, udder texture, strength of udder attachment, depth of udder, and temperament. Estimates of genetic correlations of type traits with production were inconclusive, owing to lack of confidence in the components of the correlation coefficient.

Phenotypic correlations between 26 type traits and production were estimated. A few other phenotypic correlations thought to be of interest were also estimated. The highest correlation found was between fat and milk production. Type traits which had estimated phenotypic correlations greater than .15 with production were dairy character, withers, depth of barrel, udder shape, depth of udder, and feeding habits. All of these correlations were less than .21.

Selection indexes for breeding value for milk production were computed, utilizing seven variables having the highest heritabilities and phenotypic correlations with production. The index using all seven variables was found to have a correlation with actual breeding value of .58. Using milk production alone to estimate breeding value had a correlation with breeding value of .53. Since selection based on only milk production is nearly as accurate as using seven variables, it appears that in any practical situation selection for milk production could be based on milk production alone.

The purpose of type classification should be to increase the accuracy of selecting dairy cows for greater production. Type classification will accomplish this only if the heritabilities are sufficiently large enough and the genetic and phenotypic covariances are of such relative magnitude that indexes involving type traits will be more accurate in predicting breeding value for production than will production alone. In New York in 1953, the Extension Division of the Animal Husbandry Department of Cornell University began a program of objective rating of type traits to see if it would be possible to find a more accurate predictor of production than was available.

The purpose of this study was to estimate the heritability of these traits and the phenotypic and genetic covariance of each with milk and fat production. These estimates were used to investigate the relative importance of these traits in selecting for production.

SOURCE AND DESCRIPTION OF THE DATA

The data included in this study were individual type appraisal records taken by extension personnel who rated a complete herd at one time. Management traits were rated by the herd manager. From 1953 to 1958 approximately

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25,000 records for all breeds were obtained. Genetic correlations and heritabilities were estimated on 842 daughter-dam pairs in 178 herds in the Holstein-Friesian breed that fulfilled the following conditions:

1. 2×, 305-day, M.E. records on both daughter and dam were available in the New York DHIA Central Processing Laboratory.
2. The above records were made in the same year the herd was type appraised.
3. There were at least two pairs in a herd.

The number of pairs per herd varied from two to 20. Phenotypic correlations were estimated from 5,763 records in 419 herds.

The original rating for each trait was not necessarily linear. In such cases, the rating was transformed to make the ranking linear. For example, depth of udder was rated: deep 1; intermediate 2, shallow 3; and too deep 4. This ranking was transformed to: shallow 1; intermediate 2; deep 3; and too deep 4. All records were on IBM punch cards. A sample of the appraisal form is presented by Trimberger (8).

There may be some question as to the reliability of the observations taken by the herd-manager. It is felt, however, that such observations were not subject to error more than observations reported by the extension personnel. Since there is widespread interest in the New York system of type appraisal, it is felt that all possible characteristics should be reported to present a more complete picture of the importance or lack of importance of the individual characteristics. The possible source of error must be considered by everyone who wishes to apply the results of the analysis.

ANALYSIS AND DISCUSSION OF RESULTS

Heritabilities and genetic correlations were calculated on a within-herd year basis. The heritabilities (h^2) were estimated as twice the regression of daughter's record on dam's record. The genetic correlations were computed by both methods proposed by Hazel (2),

$$g_{ij} = \frac{\frac{1}{2}(C_{ij}+C_{ji})}{\sqrt{C_{ii}C_{jj}}} \tag{1}$$

$$\gamma_{ij} = \sqrt{\frac{C_{ij}C_{ji}}{C_{ii}C_{jj}}} \tag{2}$$

where C_{ij} and C_{ji} are the estimates of the genetic covariance between traits i and j, and C_{ii} and C_{jj} are the estimates of the genetic variance of traits i and j, respectively.

If the signs of C_{ij} and C_{ji} are different, γ_{ij} should not be estimated. If either C_{ii} and C_{jj} are negative, neither estimate of the genetic correlation should be computed.

Standard errors of the heritability estimates were estimated as:

$$2\sqrt{\frac{\frac{\sigma_y^2}{\sigma_x^2} - \frac{(\hat{h}^2)^2}{4}}{N-H-1}}$$

where σ_y^2 is the error variance of daughters and σ_x^2 is the error variance of dams, N is the total number of pairs, and H is the number of herds. This computational formula, derived from the usual estimated standard error of a regression coefficient, was suggested by VanVleck *et al.* (11).

Phenotypic correlations were estimated on a within-herd year basis. The components of variance were also estimated for the one-way classification model, where classes were herd-year groups. The variances of these components were calculated according to the formulas given by Searle (4). The estimates of the phenotypic and genetic covariances were used to construct indexes for estimated breeding value of milk production.

Heritabilities. The heritability estimates of fat and milk production obtained in this study were .42 and .40, respectively. These estimates seem high when compared with those found by other investigators (5, 6, 7, 9, 10). This difference may be due to the complete confounding of herd and year effects in this study. Removal of the herd effect automatically removed the effect due to year. Tabler and Touchberry (6) obtained estimates of .24 and .27 for fat and milk yield, respectively, but without restricting the daughter and dam records to the same year. The estimate of heritability of depth of barrel, .33, is in accord with that found by Touchberry (7) for paunch girth, .26. The heritability estimates of leg and feet characteristics are similar to those found by Grieve (1).

As can be seen from Table 1, the management traits which have heritability estimates significantly greater than zero at the .05 level of significance are temperament .40, milking quality .24, and milk leak .11. It is interesting to note that the estimated heritabilities of the incidence of mastitis, ketosis, and breeding trouble are very low.

The only heritability estimate of a body characteristic whose confidence interval does not include zero is depth of barrel, .33. The leg traits and pin bone width have low heritability estimates. Among the udder characteristics with relatively high heritability estimates are: udder texture .28; strength of rear udder attachment .30; depth of udder .22; udder quartering .18, and strength of fore udder attachment .16. Other udder traits have low estimates of heritability.

Genetic correlations. The estimates of genetic correlations obtained using the methods described by Hazel (2) are listed in Table 1. The variance associated with a genetic correlation is not known; consequently, a confidence interval for a genetic correlation also is unknown. Reeve (3), however, gives an approximate procedure for estimating the variance of the estimate of a genetic correlation. He also states that if heritability is assumed to be small the variance increases roughly in proportion to $(1-\gamma^2)$ as the genetic correlation (γ^2) decreases. An estimate of the genetic correlation between two traits, when one or both have a heritability of zero is, of course, meaningless. Since the estimate of a genetic correlation is a function of the correlations between traits of the daughter-dam pair, sampling errors in estimating these correlations can markedly affect the estimate of the genetic correlation.

The simple correlations between the type traits of the daughters and the

TABLE 1

Heritability estimates and genetic correlations with production for 27 type traits

Trait	Degrees of freedom	Heritability estimate	Standard error of heritability estimates	Genetic correlation: With fat (1)[a]	With fat (2)[a]	With milk (1)[a]	With milk (2)[a]
Production and body characteristics							
Dairy character	664	.10	.080	.27	*	.98	.95
Head	664	.15	.092	.06	*	.24	*
Shoulder	664	.10	.062	−.24	−.14	.20	*
Withers	249	.16	.118	.35	.35	.22	.03
Hind legs (side)	664	.08	.078	.12	*	−.05	*
Hind legs (rear)	664	.04	.077	.34	.18	.14	*
Pasterns	664	.12	.075	.19	.19	.32	*
Rear leg movement	649	.07	.057	−.30	−.29	−.10	*
Depth of barrel	525	.33	.115	.30	.27	.24	.13
Pin bone width	652	.05	.096	.40	.39	.30	*
Fat yield	664	.42	.074	.....	.....	.....	.....
Milk yield	664	.40	.077	.77	.77	.....	.....
Udder characteristics							
Udder shape (rear)	415	.04	.109	−.54	−.54	.29	*
Udder shape (fore)	487	−.05	.094	*	*	*	*
Udder texture	523	.28	.069	−.06	.03	.14	*
Depth	464	.22	.096	−.11	*	.14	*
Levelness	464	.09	.072	−.30	*	.14	*
Strength of attachment (rear)	664	.30	.065	.05	*	.02	*
Strength of attachment (fore)	664	.16	.060	.02	.19	.12	*
Udder quartering	663	.18	.078	.00	*	−.02	*
Teat length (rear)	652	−.09	.090	*	*	*	*
Teat length (fore)	651	.05	.088	.42	*	.66	*
Management traits							
Temperament	661	.40	.085	.16	.09	.08	.05
Feeding habits	661	.02	.084	1.11	1.05	1.24	1.04
Mastitis	661	.05	.060	−.12	−.02	.44	*
Ketosis	659	−.03	.039	*	*	*	*
Breeding trouble	661	.03	.060	.29	*	.32	*
Milking quality	660	.24	.078	.11	.11	.10	*
Milk leak	660	.11	.056	.21	.16	−.25	−.17

* Indicates the estimate is imaginary.

[a] (1) Estimated as $\frac{\frac{1}{2}(C_{ij} + C_{ji})}{\sqrt{C_{ii}C_{jj}}}$ (2) Estimated as $\sqrt{\frac{C_{ij}C_{ji}}{C_{ii}C_{jj}}}$

where C's are estimates of population covariances.

production of the dams were found to be between −.06 and .12; the corresponding correlations between production of the daughters and type traits fo the dams were between −.05 and .07. Only correlation coefficients with absolute values greater than .08 can be considered significantly different from zero at the .05 significance level. In this study only six of these correlations were equal to or exceeded .08. None of the correlations of type traits of the dams with production of the daughters was as great as .08. If the correlations of the same traits between dams and daughters are subjected to similar examination only milking quality .12; depth of barrel .12; udder texture .18; strength of rear udder attachment .18; strength of fore udder attachment .10; udder quartering .09; withers .09; temperament .18; and depth of udder .10 exceed the .08 point. At least one or more of the components of the genetic correla-

tions estimated in this study can not be said to be different from zero. This indicates that the estimates of genetic correlations of production with type traits listed in Table 1 can not be said to be significantly different from zero.

The genetic correlation found in this study between milk and fat yield, .77, agrees with estimates found by other investigators (6, 7, 9). All of the components making up this estimate are significantly greater than zero.

Phenotypic correlations. The estimates of the phenotypic correlations obtained in this analysis are listed in Table 2. Estimates which have an absolute value greater than .03 can be said to be significantly different from zero at the .05 level. The highest value, .87, is the correlation between milk and fat

TABLE 2

Estimated phenotypic correlations and components of variance

Trait	Degrees of freedom[a]	Phenotypic correlation with Fat	Milk	D.C.[b]	M.Q.[c]	D.U.[d]	Components of variance[e] $\hat{\sigma}_h^2$	$\hat{\sigma}_e^2$
Production and body characteristics								
Dairy character	5344	.184	.209	.....	−.024	.169	.0365	.2425
Head	5344	.081	.080	.194	*[f]	*	.0149	.1852
Shoulder	5344	−.071	−.077	.078	*	*	.0266	.2314
Withers	2160	.154	.150	*	*	*	.0395	.3194
Hind legs (side)	5344	.015	.021	*	*	*	.0365	.3144
Hind legs (rear)	5344	−.008	.004	*	*	*	.0361	.2827
Pasterns	5344	−.024	−.033	−.051	*	*	.0549	.3176
Rear leg movement	5172	−.032	−.033	*	*	*	−.0022	.0321
Depth of barrel	4357	.134	.158	.186	−.056	.312	.0387	.2692
Pin bone width	5187	.046	.054	*	*	*	.0562	.2813
Fat yield	5344	.....	.873	.184	.039	.136	3,037	6,094
Milk yield	5344	.873	.....	.209	.006	.191	1,803,400	4,535,700
Udder characteristics[g]								
Udder shape (rear)	3913	.147	.177	*	*	*	.0458	.3823
Udder shape (fore)	4836	.111	.093	*	*	*	.0310	.3685
Udder texture	4342	.000	−.026	*	.227	−.156	.0553	.2545
Depth of udder	3832	.136	.191	.169	−.158	*	.0634	.3617
Levelness	3831	.011	−.017	*	.113	−.172	.0626	.5125
Strength of attachment (rear)	5342	−.021	−.044	*	*	*	.0474	.4465
Strength of attachment (fore)	5342	−.049	−.088	*	*	*	.0592	.5157
Udder quartering	5342	.015	.003	*	*	*	.0527	.3590
Teat length (fore)	5190	.002	−.022	*	*	*	.0280	.2239
Management traits								
Temperament	5328	.052	.049	.000	−.002	−.015	.0050	.1639
Feeding habits	5328	.158	.177	*	*	*	.0718	.2096
Mastitis	5331	.055	.004	*	.114	*	.0133	.1553
Ketosis	5316	−.045	−.063	*	*	*	.0017	.0545
Breeding trouble	5338	−.068	−.073	*	*	*	.0050	.0980
Milking quality	5326	.039	.006	−.024	*	*	.0643	.3719
Milk leak	5325	.010	.004	*	*	*	.0008	.0499

[a] Degrees of freedom are for correlations with milk and fat production and for the components of variance.

[b] D.C. = Dairy character.

[c] M.Q. = Milking quality.

[d] D.U. = Depth of udder.

[e] Components of variance were estimated for the model $Y_{ij} = \mu + h_i + e_{ij}$, where Y_{ij} is the observation on the j^{th} cow in the i^{th} herd-year and the h_i's and e_{ij}'s are independently distributed with zero means and variances σ_h^2 and σ_e^2, respectively.

[f] * Indicates the estimate was not computed.

[g] The estimated phenotypic correlation between mastitis incidence and udder texture = .114.

production. This is similar to values previously reported by Tyler and Hyatt (9) and Tabler and Touchberry (5).

A few phenotypic correlations other than those with production also were computed. Some of the more interesting ones are those between dairy character and body traits. Head, depth of barrel, and depth of udder apparently are positively correlated with dairy character. This could be due to the influence these traits have on the rating given dairy character by the rater. Udder texture, levelness of udder, and mastitis incidence seem to be positively correlated with milking quality. Depth of udder appears to have a significant negative correlation with udder texture, levelness of udder, and milking quality. Depth of udder, however, has a positive phenotypic correlation with depth of barrel.

All type traits have relatively low phenotypic correlations with production. Some of the higher estimates are those for dairy character, withers, depth of barrel, udder shape, depth of udder, and feeding habits with production. Of these, only depth of barrel has a heritability estimate greater than .15. This would suggest that selection for these traits of the dam would not have a very large effect on production of the daughter. Accordingly, only those traits with relatively high heritabilities and phenotypic correlations with production were used in constructing selection indexes for breeding value of production of the dams. Dairy character was included as an exception, since it is thought by many to be indicative of producing ability.

Selection indexes. The indexes used in estimating breeding value of a cow were of the form:

$$I = \sum_{i=1}^{n} b_i X_i,$$

where I = estimate of breeding value for milk production,
X_1 = fat production,
X_2 = milk production,
X_3 = dairy character,
X_4 = depth of barrel,
X_5 = depth of udder,
X_6 = milking quality, and
X_7 = temperament, all observed on the cow.

The set of equations used to estimate the b's were of the form:

$$b_1 \sigma^2_{X_1} + b_2 \sigma_{X_1X_2} + \ldots + b_n \sigma_{X_1X_n} = \sigma_{X_1g}$$

$$b_1 \sigma_{X_1X_2} + b_2 \sigma^2_{X_2} + \ldots + b_n \sigma_{X_2X_n} = \sigma_{X_2g}$$

$$\vdots$$

$$b_1 \sigma_{X_1X_n} + b_2 \sigma_{X_2X} + \ldots + b_n \sigma^2_{X_n} = \sigma_{X_ng},$$

where $\sigma^2_{X_i}$ = variance of trait X_i;
$\sigma_{X_iX_j}$ = phenotypic covariance between traits X_i and X_j; and,
σ_{X_ig} = the genetic covariance between trait X_i and breeding value for milk production.

Estimates of the variances and covariances found in this study were substituted into the equations and the equations solved for the b's. The estimates of phenotypic variances used in the index equations were those obtained from the analysis of 5,763 records. Which estimates of parameters to use in index equations is an unanswered problem. In this study the estimates associated with the larger number of degrees of freedom were used. The index equation involving all seven variables was then estimated to be:

$$I = -2.06X_1 + .35X_2 + 623.11X_3 - 217.89X_4 - 125.10X_5 + 79.82X_6 + 66.48X_7.$$

These b's actually appear to give an exaggerated importance to some characteristics. The magnitudes of X_3, X_4, X_5, X_6, and X_7 are in the range one to four, whereas X_2 is in thousands and X_1 in hundreds. The standard partial regression coefficients give a much clearer picture of the relative importance of these traits. The partial regression coefficients and the standard partial regression coefficients for the seven variables are shown in Table 3.

TABLE 3[a]

Partial and standard partial regression coefficients for the index with seven variables

Trait	b	b'
Fat production	−2.06	−.08
Milk production	.35	.35
Dairy character	623.11	.14
Depth of barrel	−217.89	−.05
Depth of udder	−125.10	−.04
Milking quality	79.82	.02
Temperament	66.48	.01

[a] b = Partial regression coefficient; b' = standard partial regression coefficient.

The standard partial regression coefficients indicate that the most important contribution to the index is made by milk production. Dairy character seems to be relatively more important than the five other variables. A probable reason for the negative coefficient associated with fat production is its high correlation with milk production. A similar reason might be given for the coefficients of depth of barrel and depth of udder, since their relation to dairy character seems to be somewhat important (see Table 2).

The correlation between the index and actual breeding value is measured by

$$R = \sqrt{\frac{\sum_{i=1}^{n} b_i \sigma_{X,g}}{\sigma_g^2}}$$

as given by Hazel (2). Table 4 presents R for indexes having various combinations of the independent variables. Including all seven independent variables gives $R = .58$. The use of milk production alone in the equation gives $R = .53$. Since the genetic progress is proportional to R, a method of comparing the relative importance of various indexes is to express the efficiency of the indexes as the ratio of one R to another in percentages. Taking the R for milk as a

base, the other R's are expressed as a percentage of the R for milk production in Table 4. This comparison shows that if the estimates of genetic and phenotypic parameters included in the equations are actually correct, the index using seven variables is about 10% more effective in selecting for milk production than the index including only milk production. This suggests that selection

TABLE 4[a]

Partial regression coefficients, R_{ij}, and R_j expressed as a percentage of the R for milk production alone for various combinations of traits used in selection indexes to estimate breeding value for milk production

Index No. $=j$	Fat	Milk	Dairy character	Depth of barrel	Depth of udder	Milking quality	Temperament	R_j[b]	Percent[c]
1	−2.06	.35	623.11	−217.89	−125.10	79.82	66.48	.5791	109.87
2	−2.05	.35	622.26	−216.92	−126.38	79.52	.....	.5789	109.84
3	−1.99	.35	622.81	−218.99	−137.51	.....	65.68	.5778	109.64
4	−2.05	.35	534.46	.....	−170.19	81.44	62.14	.5735	108.82
5	−1.82	.37	.....	22.12	−117.05	78.54	57.19	.5345	101.42
6	6.20	.....	662.82	−202.37	44.39	60.67	77.41	.4981	94.52
7	.....	.29	617.10	−217.43	−117.26	72.18	62.81	.5758	109.25
8	−1.90	.34	507.14	.....	.....	106.24	68.14	.5681	107.79
9	.....	.29	528.67	.....	−162.27	73.88	58.86	.5702	108.19
10	.....	.28	614.81	−256 39	.....	63.97	67.23	.5728	108.69
11	−1.97	.35	621.98	−218.02	−138.73	.....	.....	.5773	109.54
12	.....	.28	502.93	.....	.....	98.14	64.50	.5648	107.17
13	.....	.....	754.38	.....	.....	109.23	136.17	.3103	58.88
14	.....	.30	.....	.....	.....	87.80	58.18	.5291	100.40
15	.....	.28	499.66	.....	.....	.....	64.19	.5627	106.77
16	.....	.28	502.36	.....	.....	98.05	.....	.5644	107.10
17	.....	.28	499.09	.....	.....	.....	.....	.5625	106.74
18	6.83	.....	.....	.....	.....	.....	.....	.4355	82.63
19	.....	.30	.....	.....	.....	.....	.....	.5270	100.00
20	.....	.....	751.08	.....	.....	.....	.....	.3025	57.40
21	.....	.....	.....	175.64	.....	.....	.....	.0738	14.00
22	.....	.....	.....	.....	91.46	.....	.....	.0461	8.75
23	.....	.....	.....	.....	.....	94.26	.....	.0461	8.75
24	.....	.....	.....	.....	.....	.....	135.88	.0461	8.75

[a] The values listed for each row are the coefficients for the traits included in that particular index. ..... indicates the trait was not used in that index.

[b] $R_j = \sqrt{\frac{\sum_{i=1}^{n} b_i \sigma_{X_i g}}{\sigma_g^2}}$, where σ_g^2 is the genetic variance of milk production.

[c] R_j expressed as a percentage of the R for the index using only milk production, R_{19}.

on the basis of milk production — which is much easier — would be nearly as effective as selection based on all seven variables. Using the other variables singly to predict breeding value for milk production, as shown in the table, is not as effective as using milk alone. Fat production, owing to its high correlation with milk production, appears to be more effective in selecting for milk production than are any of the five other variables. All others, except for dairy character, which is about half as efficient as milk production, seem not to be very effective for selecting for breeding value for milk production.

REFERENCES

(1) Grieve, D. G., Rennie, J. C., and Raithley, G. E. Heritability of Various Characteristics of Body Conformation and Their Association with Production of Holstein-Friesian Cows in Canada. J. Dairy Sci., 41: 748. 1958.

(2) HAZEL, L. N. The Genetic Basis for Constructing Selection Indexes. Genetics, 28: 476. 1943.

(3) REEVE, E. C. The Variance of the Genetic Correlation Coefficient. Biometrics, 11: 357. 1955.

(4) SEARLE, S. R. Matrix Methods in Components of Variance and Covariance Analysis. Ann. Math. Stat., 27: 737. 1956.

(5) TABLER, K. A., AND TOUCHBERRY, R. W. Selection Indices Based on Milk and Fat Yield, Fat Per cent, and Type Classification. J. Dairy Sci., 38: 1155. 1955.

(6) TABLER, K. A., AND TOUCHBERRY, R. W. Selection Indices for Milk and Fat Yield of Holstein-Friesian Dairy Cattle. J. Dairy Sci., 42: 123. 1959.

(7) TOUCHBERRY, R. W. Genetic Correlations Between Five Body Measurements, Weight, Type and Production in the Same Individual Among Holstein Cows. J. Dairy Sci., 34: 242. 1951.

(8) TRIMBERGER, G. W. Dairy Cattle Judging Techniques. pp. 289-290. Prentice-Hall, Inc., Englewood Cliffs, New Jersey. 1958.

(9) TYLER, W. J., AND HYATT, GEORGE J. The Heritability of Milk and Butterfat Production and Percentage of Butterfat in Ayrshire Cattle. J. Animal Sci., 6: 479. 1947. (Abstract.)

(10) TYLER, W. J., AND HYATT, GEORGE J. The Heritability of Official Type Ratings and Butterfat Production of Ayrshire Cows. J. Dairy Sci., 31: 63. 1948.

(11) VANVLECK, L. D., SEARLE, S. R., AND HENDERSON, C. R. The Number of Daughter-Dam Pairs Needed for Estimating Heritability. J. Animal Sci., 18: 1466. 1959. (Abstract.)

EFFECT OF PROMAZINE HYDROCHLORIDE AND CHLORPROMAZINE HYDROCHLORIDE ON THE MOTILITY AND FERTILITY OF BOVINE SEMEN

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SUMMARY

Promazine hydrochloride (Sparine) and chlorpromazine hydrochloride (Thorazine) were incorporated in 20% egg yolk extenders of the CU-16 type at levels as high as 2,000 μg. per milliliter of extender. None of the levels of promazine tested improved sperm survival, and the highest levels of both drugs were spermicidal ($P < 0.01$). Survival of spermatozoa was improved by the replacement of sulfanilamide (SA) with 200 μg. chlorpromazine per milliliter in CU-16 extender, but chlorpromazine was only partially effective in replacing SA in CUE extender. The same level of chlorpromazine was slightly spermicidal when added to a 50% yolk-citrate-sulfanilamide extender, and markedly spermicidal when added to heated skimmilk.

The 60- to 90-day nonreturns for semen extended in the CU-16 extender, modified by replacing SA with 200 μg. of chlorpromazine per milliliter, averaged 73.9% compared to 70.3% for the control 50% yolk-citrate-sulfanilamide extender. Subsequently, this modified CU-16 extender was compared with the sulfanilamide-containing standard CU-16 and CUE extenders. The number of first services and 60- to 90-day per cent nonreturns for the three extenders, respectively, were 4,796 and 73.7, 4,871 and 74.9, and 4,650 and 74.9 ($P > 0.05$). While all three extenders gave highly acceptable fertility, there appears to be no advantage in replacing sulfanilamide with chlorpromazine in the extenders used in these experiments.

Tranquilizers, such as promazine and chlorpromazine, have become a widely administered group of compounds. Little is known about their effects on reproduction, although the sedative effects of chlorpromazine have been utilized to facilitate examination of the reproductive organs of the bull (9) and the collection of semen by electro-ejaculation (6). Several of these compounds have been reported by Himwich (7), Kopera and Armitage (8), and others to affect cellular metabolism in general, as well as to cause depression of the cortical centers. Both effects might influence spermatogenesis and spermatozoa in vivo, and any general depressant of cellular metabolism might be useful in prolonging sperm survival in vitro. Burton *et al.* (2) found that promazine and chlorpromazine could prolong artificially elevated levels of diphosphopyridine nucleotide in the liver, a compound present in sperm and involved in fructolysis. A brief qualitative report by Manente (11) suggested that bull sperm survival may be increased by the addition of chlorpromazine, whereas unpublished observations by Lutwak-Mann and Mann (10) indicated that chlorpromazine may abolish metabolism and motility of ram spermatozoa. In view of the widespread use of tranquilizers and the limited information available in the literature, studies of the effects of promazine hydrochloride and chlorpromazine hydrochloride upon the survival and fertility of bovine spermatozoa were undertaken.

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EXPERIMENTAL PROCEDURE

The promazine hydrochloride used was sold under the trade name Sparine, and the chlorpromazine hydrochloride as Thorazine. For the sake of brevity in this paper they are referred to as promazine and chlorpromazine. The basic extenders, to which varying amounts of these compounds were added, were of the CU-16 and CUE types described by Foote *et al.* (3, 4), a 50% yolk citrate extender, and skimmilk prepared according to the report by Almquist *et al.* (1). The standard CU-16 and CUE extenders contain 20 parts of egg yolk mixed with 80 parts of a buffer solution composed of glucose, glycine, sodium citrate, and other salts. These extenders also contained 0.24% sulfanilamide (SA) and 1,000 units of penicillin and 1,000 μg. of dihydrostreptomycin per milliliter.

For both the storage and fertility studies semen was obtained from bulls ejaculated at weekly intervals in the stud of the New York Artificial Breeders' Cooperative, Inc. The semen was extended to give ten million motile spermatozoa per milliliter of extender, which resulted in average extension rates slightly in excess of 1:100.

In laboratory trials subsamples were removed after one, four, eight, and 12 days of storage at 5° C., and examined microscopically in a stage incubator at 37° C. for the percentage of motile spermatozoa. The pH of the extenders and of the extended semen was measured in a Beckman model G pH meter.

In the fertility trials which followed the laboratory studies the design was such that treatments were not confounded with bulls, time trends, or inseminators. With this design an unbiased test of the significance of treatment differences can be made. The fertility was measured as the percentage of first-service cows not returning for reinsemination within 60 to 90 days after the initial service. The analyses of variance of these data were done according to the procedures outlined by Henderson (5).

RESULTS AND DISCUSSION

Laboratory studies. In the first experiment promazine and chlorpromazine were added to the CU-16 extender, which was modified by replacing sulfanilamide with 0, 20, 200, and 2,000 μg. of tranquilizing drugs per milliliter of extended semen. The highest level of each drug tested was definitely harmful to the spermatozoa ($P < 0.01$). Levels up to 200 μg. per milliliter either were beneficial or without effect. A second experiment was conducted using intermediate levels of tranquilizers, and the results of these two experiments are shown in Table 1. After eight days of storage the 100-μg. and 200-μg. levels of chlorpromazine were superior to the control ($P < 0.01$). Similar levels were found by Burton *et al.* (2) to produce effects on the diphosphopyridine nucleotide content of liver. Promazine showed no significant beneficial effects, and further studies with this compound were discontinued after a study with 30 ejaculates of semen. The initial pH of all the extenders was approximately 6.9, and after 12 days of storage the pH of the extended semen ranged from

TABLE 1

Percentage of motile spermatozoa in modified CU-16 extender containing promazine or chlorpromazine but no sulfanilamide

Expt.	Days stored at 5° C.	Control, 0 drug	Promazine (μg/ml)				Chlorpromazine (μ/ml)			
			20	100	200	2000	20	100	200	2000
			(% motile spermatozoa)							
I (10 ejac.)	1	61	59	....	54	37	58	....	56	35
	4	56	56	....	52	14	56	....	53	23
	8	33	31	....	41	0	37	....	42	10
	12	19	18	....	26	0	24	....	30	4
	Mean	42	41	....	43	13	44	....	45	18
II (20 ejac.)	1	58	60	60	58	....	60	58	62	....
	4	54	55	56	56	....	57	54	58	....
	8	30	30	36	36	....	38	47	49	....
	12	13	13	16	16	....	15	27	34	....
	Mean	38	39	42	41	....	42	47	51	....

6.59 to 6.68. Therefore, pH would not appear to be a factor affecting sperm survival in these experiments.

Next, chlorpromazine was added to the CUE extender in comparison with CU-16. Sulfanilamide was included in one series of treatments and omitted from an otherwise duplicate series. The results are shown in Table 2. Over

TABLE 2

Percentage of motile spermatozoa in CU-16 and CUE extenders with and without chlorpromazine and sulfanilamide
(Mean of ten ejaculates)

Extender	Days stored at 5° C.	No sulfanilamide			300 mg. % sulfanilamide		
		Chlorpromazine (μg/ml)			Chlorpromaizne (μg/ml)		
		0	200	400	0	200	400
				(% motile spermatozoa)			
CU-16	1	61	60	59	57	59	57
	4	54	55	53	53	52	51
	8	37	47	39	35	37	34
	12	22	34	27	30	27	24
	Mean	44	49	44	44	44	42
CUE	1	56	56	59	62	61	55
	4	43	46	52	56	53	53
	8	21	28	33	46	42	38
	12	9	24	27	35	25	26
	Mean	32	39	43	50	45	43

the 12-day storage period chlorpromazine improved sperm survival in the absence of sulfanilamide, but not in the presence of sulfanilamide. The 200-μg. level was nonsignificantly higher than the 400-μg. level ($P > 0.05$). The best survival was obtained in the CU-16 extender modified by replacing the sulfanilamide with 200 μg. per milliliter of chlorpromazine, and in the standard CUE extender containing sulfanilamide but no chlorpromazine. These findings were supported by the results shown in Table 3, which includes a 50% citrate-sulfanilamide-yolk medium (2.9 CSAY) commonly used by artificial breeding organizations. Separate analyses of the experiments, shown in Tables 2 and 3,

TABLE 3

Percentage of motile spermatozoa in modified CU-16, modified CUE, and 2.9 CSAY extenders containing sulfanilamide, and 0 and 200 μg. of chlorpromazine per milliliter (Mean of ten ejaculates)

Days stored at 5° C.	Control[a]	Levels of chlorpromazine (μg/ml) in:					
		CU-16		CUE		2.9 CSAY[b]	
		0	200	0	200	0	200
		(% motile spermatozoa)					
1	65	57	63	59	62	61	58
4	60	59	61	59	59	58	57
8	55	57	56	58	56	57	49
12	50	41	40	48	39	38	28
Mean	58	54	55	56	54	54	48

[a] Control was the modified CU-16 containing 200 μg. of chlorpromazine per milliliter but no sulfanilamide. All other treatments contained sulfanilamide.

[b] Consisted of a buffer containing 2.9% of sodium citrate dihydrate and 0.6% of sulfanilamide mixed in equal parts with egg yolk.

and a single analysis combining the treatments included in both experiments, revealed the following:

1. Replacing the sulfanilamide in the CU-16 extender with 200 μg. per milliliter of chlorpromazine significantly improved sperm survival over a 12-day storage period ($P < 0.01$).

2. Replacing the sulfanilamide in the CUE extender with chlorpromazine or its inclusion with sulfanilamide in the extender reduced the percentage of motile spermatozoa at 12 days of storage ($P < 0.01$).

3. Addition of chlorpromazine to the 2.9 CSAY extender reduced sperm survival ($P < 0.01$).

The same extenders as those shown in Table 3 were used for storing semen in tubes and in ampules at both 5 and 25° C. Both types of storage containers gave similar results, but the average motility of the spermatozoa was lower at 25° C. than at 5° C., particularly when sulfanilamide was absent.

An experiment with CU-16 extender, in which 100-μg. and 200-μg. levels of chlorpromazine per milliliter were combined with 0, 0.15, and 0.30% of sulfanilamide, showed no difference between levels of the tranquilizer. However, a linear reduction in sperm survival resulted as the concentration of sulfanilamide increased. The percentage of motile spermatozoa at the twelfth day of storage averaged 36, 30, and 23 for the 0, 0.15, and 0.30% levels of sulfanilamide, respectively.

Heated skimmilk is commonly used for extending bovine semen (1), and the effects of adding chlorpromazine to this extender are shown in Table 4. The differences among the three extenders were highly significant ($P < 0.01$), indicating better survival of the spermatozoa in the modified CU-16 extender than in skimmilk, and a toxic effect of chlorpromazine in skimmilk.

Fertility trials. Because the modified CU-16 extender, in which the sulfanilamide was replaced with 200 μg. of chlorpromazine per milliliter of extender, seemed promising for the preservation of bull semen used in artificial insemination, a fertility trial, comparing it with the 50% citrate-sulfanilamide–buf-

TABLE 4

Percentage of motile spermatozoa in heated skimmilk with and without chlorpromazine (Mean of ten ejaculates)

Days stored at 5° C.	Control (Modified CU-16)	Heated skimmilk	Heated skimmilk plus 200 μg. chlorpromazine per milliliter
		(% motile spermatozoa)	
1	61	60	49
4	60	55	15
8	49	32	0
12	32	7	0
Mean	50	38	16

fered yolk (2.9 CSAY) then in standard use by the New York Artificial Breeders' Cooperative, Inc., was conducted in April and May, 1958. This first trial was conducted on a small scale, in view of the work of Lutwak-Mann and Mann (10) indicating that under certain conditions chlorpromazine may damage spermatozoa. Four bulls were used. Their average 60- to 90-day nonreturn rate in routine service just prior to and following the experiment was 70%. The results of the experiment were as follows:

Extender	No. of first services	60- to 90-day per cent nonreturns
Control 2.9 CSAY	1497	70.3
Modified CU-16	1324	73.9

The difference between treatments was not statistically significant ($P > 0.05$), although all four bulls had higher nonreturn rates in the experimental extender.

In March, 1959, a second field trial was conducted in which the modified CU-16 extender, containing chlorpromazine but no sulfanilamide, was compared with the standard CU-16 and CUE extenders containing sulfanilamide. Twelve Holstein bulls were used, and the results are shown in Table 5. The

TABLE 5

Number of first services and percentage of 28- to 35-day and 60- to 90-day nonreturns in Trial 2

	CUE	CU-16	Modified CU-16, SA replaced with chlorpromazine
No. of first services	4,650	4,871	4,796
28- to 35-day per cent nonreturns	84.6	85.3	84.0
60- to 90-day per cent nonreturns	74.9	74.9	73.7
Decrease in per cent nonreturns	9.7	10.4	10.3

three extenders gave similar nonreturn rates 28 to 35 days and 60 to 90 days after insemination ($P > 0.05$). These nonreturn rates are higher than those obtained with the 2.9 CSAY control in the first fertility trial, but are con-

sistent with the generally higher fertility levels previously reported for the CUE and CU-16 extenders by Foote *et al.* (4).

It is concluded from these studies that the modified CU-16 extender containing chlorpromazine but no sulfanilamide may have a fertility advantage over the 2.9 CSAY extender but none over the standard CU-16 extender containing sulfanilamide. At the same time, however, it is important to note that levels of chlorpromazine as high as 200 μg. per milliliter of CU-16 extender did not reduce fertility. The regular CUE and CU-16 extenders appear to be equal in maintaining high nonreturn rates. The results of this trial added to those previously reported (4) give a total of 10,930 first inseminations and 75.9% 60- to 90-day nonreturns for the CUE extender, and 11,016 first services and 75.5% nonreturns for the CU-16 extender.

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REFERENCES

(1) Almquist, J. O., Flipse, R. J., and Thacker, D. L. Diluters for Bovine Semen. IV. Fertility of Bovine Spermatozoa in Heated Homogenized-Milk and Skimmilk. J. Dairy Sci., 37: 1303. 1954.

(2) Burton, R. M., Kaplan, N. O., Goldin, A., Leitenberg, M., Humphreys, S. R., and Sodd, M. A. Effects of Reserpine and Promazine on Diphosphopyridine Nucleotide Synthesis in Liver. Science, 127: 30. 1958.

(3) Foote, R. H., and Bratton, R. W. Survival of Bovine Spermatozoa Stored at 5 and 25° C. in Extenders Containing Varying Levels of Egg Yolk, Glucose, Glycine, Citrate, and Other Salts. J. Dairy Sci., 43: 1322. 1960.

(4) Foote, R. H., Gray, Linda C., Young, D. C., and Dunn, H. O. Fertility of Bull Semen Stored Up to Four Days at 5° C. in 20% Egg Yolk Extenders. J. Dairy Sci., 43: 1330. 1960.

(5) Henderson, C. R. The Design and Analysis of Animal Husbandry Experiments. Dept. of Animal Husbandry, Cornell University. (Mimeo.) 1955.

(6) Herrick, J. B. An Aid to Collection of Semen by Electroejaculation, Chlorpromazine Hydrochloride. Vet. Med., 53: 473. 1958.

(7) Himwich, H. E., ed. Tranquilizing Drugs. Am. Assoc. Adv. Sci., Publ. No. 46. Washington, D. C. 1957.

(8) Kopera, J., and Armitage, A. K. Comparisons of Some Pharmacological Properties of Chlorpromazine, Promethazine and Pethidine. Brit. J. Pharmacol., 9: 392. 1954.

(9) Lundvall, R. L., and Campbell, R. L. Chlorpromazine Hydrochloride for the Examination of the Penis in Bulls. J. Am. Vet. Med. Assoc., 131: 86. 1957.

(10) Lutwak-Mann, C., and Mann, T. Unpublished Data. 1954. (Cited by T. Mann. Biochemical Basis of Spermicidal Activity.) Studies on Fertility, 9: 1. 1958.

(11) Manente, B. A. Survivance du Spermatozoide du Bos Taurus obtenue in Vitro avec quelques Derives de la Phenothiazine. Anesthésie et analgesie, 13: 530. 1956.

TECHNICAL NOTES

CORRELATION AND RELATIVE PRECISION OF TWO METHODS OF MEASURING THE CHLORIDE CONTENT OF MILK[1]

New tests have been developed recently for estimating the chloride content of milk (1, 4). For many years modifications of Mohr's direct titration method, in which chloride is titrated with a standardized solution of silver nitrate in the presence of potassium chromate indicator, have been in general use in this country (2). These methods have yielded values which were artificially high primarily because of interference by casein. The new potentiometric methods were developed to eliminate this source of error. The objectives of this investigation were (a) to compare the precision of a commonly used modified direct titration method (3) with the precision of one of the new potentiometric methods (1), and (b) to compare the results obtained by one of these methods with those of the other.

In comparing the precision of the two methods, composite (A.M. and P.M.) milk samples were collected at random from individual cows in the Florida Agricultural Experiment Station herd. Analyses were made in duplicate by five different operators, using both of the methods. Each operator made a preliminary trial to gain some experience with both methods. Three successive trials were then made over a 3-wk. period on ten samples of normal, fresh, whole milk. The chloride content was determined by one method one day and the other method the following day by each of the five operators. In each case, the operator was given a sample to practice on before starting on the ten samples. It was not possible for the operator to compare duplicates during the performance of the titrations.

A Beckman Model H2 pH meter with a Beckman silver electrode, Serial No. 39261, was employed in the potentiometric method. The milk was acidified with 20 ml. of 10% (w/v) potassium alum; and agitation was accomplished with a mechanically driven glass stirring rod.

Operator repeatability is shown (Table 1) for each method. Standard inter-class correlation procedures were used; estimates represented the correlation between duplicate individual-cow milk samples. Although repeatability of the potentiometric method was higher with four of five operators, and with over-all (total), differences were extremely small and must be considered unimportant. Standard errors of estimate were 0.0057 and 0.0042.

Although operators differed considerably in their previous experience with each method, these differences had no apparent effect on their abilities to make precise measurements. Results indicated that little could be gained by making duplicate determinations with either method for routine laboratory work.

TABLE 1

Operator repeatability of two methods of estimating the chloride content of milk

Operator[a]	Samples	Direct titration	Potentiometric
	(No.)	*(r)*	
A	30	0.9962	0.9970
B	30	0.9970	0.9987
C	30	0.9934	0.9985
D	30	0.9941	0.9987
E	30	0.9948	0.9915
Over-all	150	0.9948	0.9968
Standard error of estimate		0.0057	0.0042

[a] Mr. Roger Shultis, Mr. David Solger, and the authors.

To establish the relationships between single determinations by each method, 271 composite (A.M. and P.M.) samples of normal, fresh, whole milk from Holstein, Jersey, and Guernsey cows in the Florida Agricultural Experiment Station herd were analyzed. These animals were of various ages and in various stages of lactation. Samples were collected in mid-December, 1959, and mid-January, 1960. Many cows were represented twice.

Multiple covariance techniques were employed to establish total and within breed-month relationships and to test for curvilinearity of these relationships. Results are presented (Table 2).

TABLE 2

Chloride content of milk as determined by two methods

Breed	Samples	Direct titration	Potentiometric
	(No.)	*(%)*	*(%)*
Holstein	61	0.147	0.108
Jersey	154	0.128	0.089
Guernsey	56	0.133	0.092
Combined	271	0.135	0.095
Standard deviation		0.020	0.019
Correlations			
Total		0.919	
Within breed-month		0.915	

Marked differences in over-all means were noted. Breed differences were highly significant ($P < 0.01$) for both methods. Month differences (not shown) were not significant for the direct titration method, but were barely

[1] Florida Agricultural Experiment Station Journal Series, No. 1088.

significant ($P < 0.05$) for the potentiometric method. Total and within breed-month correlations were essentially the same. Variability within breed-month did not differ significantly between the two methods; standard deviations were 0.020 and 0.019. There was no evidence of a curvilinear relationship between values resulting from the two methods. Linear regressions of direct titration values on potentiometric values were 0.955 and 0.960, on a total and within breed-month basis, respectively.

Direct titration values (Mohr) could then be represented as:

$$Y = 0.0438 + 0.960\ X,$$

where Y represents the predicted value, 0.0438 the intercept, 0.960 the within breed-month regression coefficient, and X the potentiometric value. Since the endpoint for the direct titration test may vary considerably among laboratories, equation values also could vary.

On the basis of these limited data, the two tests would appear to have about the same degree of sensitivity and to take about the same amount of time to run. There is less chance of human error in the potentiometric method. All five operators agreed that the relative ease in detecting the endpoint by the potentiometric method is a point in its favor.

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REFERENCES

(1) HERRINGTON, B. L., AND KLEYN, D. H. A Potentiometric Method for the Determination of Chloride in Milk. J. Dairy Sci., 43: 1050. 1960.

(2) SANDERS, G. P. The Determination of Chloride in Milk. J. Dairy Sci., 22: 841. 1939.

(3) SHARP, P. F., AND STRUBLE, E. B. Period of Lactation and the Direct Titratable Chloride Value of Milk. J. Dairy Sci., 18: 527. 1935.

(4) WENNER, V. R. The Rapid Determination of Minerals and Ions in Milk. J. Dairy Sci., 43: 22. 1960.

OBSERVATIONS ON THE INFUSION OF PENICILLIN IN THE MAMMARY GLAND OF THE BOVINE

Antibiotics are generally administered to mastitis-infected animals via a solution or suspension manually infused into the infected quarter(s) of the udder. Current recommendations state that milk from antibiotic-treated cows should be discarded for 72 hr., or six milkings, after the last treatment. Questions have arisen concerning the possible presence or absence of antibiotics in the nontreated quarters following treatment.

Randall *et al.* (7) reported that milk from the untreated quarters of two cows showed the presence of chlortetracycline (aureomycin) and oxytetracycline (terramycin) following dosages of 426 and 852 mg. of each agent. Chlortetracycline persisted in the milk of the treated quarters for as long as 5 wk. and of the untreated quarters for a period of five days following treatment with the larger dosage levels. Following intramammary infusion of oxytetracycline ointment into two cows, the antibiotic could be detected in the milk from treated quarters for three to four days and from the untreated quarters for one day.

A recent Ohio study, in which 11 cows were infused with either 100,000 or 200,000 units penicillin (oil and aqueous carriers) per quarter, showed penicillin present in 21 out of 22 untreated quarters. All untreated quarters were negative for the presence of penicillin by 48 hr. and there were only two positive untreated quarters at 36 hr. post-infusion (2). In another Ohio study, one cow that was infused with 150,000 units penicillin into the right rear and right front quarters shed detectable amounts of penicillin in all four quarters for as long as 84 hr. (1).

Shahani (8) used a green turquoise dye mixed with a penicillin in oil preparation in intramammary infusions for the visual detection of the antibiotic in milk and found that neither the dye nor the antibiotic diffused from one quarter to another. He postulated that the dye formed a complex with the antibiotic.

An udder infusion study was conducted with ten cows (seven Brown Swiss and three Holsteins) in the University of Illinois dairy herd, to check the transfer of penicillin in aqueous solution and in oil suspensions in treated to untreated quarters. The group consisted of three mastitis-infected and seven normal cows as determined by the strip cup, California Mastitis Test, direct microscopic examination (leucocytes and identification of bacteria) standard plate counts, and examination for clinical symptoms. The cows represented various stages of lactation and production levels and averaged 38 lb. milk/day during the experimental period, with a range of 20-50 lb. milk/day. Each cow was checked and found to be negative for antibacterial substances contained in the milk, using the Arret and Kirshbaum test (3). This precaution was taken because Hollister *et al.* (4) found that four of 49 cows showed antibacterial activity in the preinjection samples. None of their cows had been subjected to antibacterial therapy for at least 3 wk. prior to the beginning of their study.

Immediately following the milking prior to the testing period, ten cows were infused in the right rear quarter with 100,000 units procaine penicillin G in oil suspension. Two weeks later the same ten cows were infused by the same veterinarian with 100,000 units procaine penicillin G in aqueous solution in the same quarter. All cows in both trials were milked with an individual quarter milker, to obtain a complete sample per quarter. Extreme care was exercised to completely rinse all equipment following the milking of each cow. The cows were milked with this unit until the milk was essentially antibiotic-free (96 hr. post-infusion). Milk samples were also taken directly from the teat as a check against possible contamination from sampling equipment.

The approved Food and Drug Administration test for the detection of antibiotics in milk (3) was conducted on all individual quarter milk samples. Penase discs were used to confirm the presence of penicillin and avoid the possibility of false readings.

As might be expected, the milk from infused quarters (aqueous and oil carriers) showed large zones of inhibition at 12 and at 24 hr. post-infusion. All of the cows treated with penicillin in oil were positive at 60 hr. following treatment. The milk from two of ten treated cows (oil carrier) was clear by 72 hr. and five of ten were clear by 96 hr. post-therapy. Unfortunately, samples were not obtained beyond the fourth day, in order to check how long the antibiotics persisted in the milk of the five remaining cows. It is not unusual to find milk positive for antibiotics beyond the recommended 72-hr. post-treatment milk disposal period (6). In the penicillin-in-water group the infused quarters of nine of ten cows were positive at 36 hr., three of ten at 48 hr., and one of ten at 60 hr. The milk from all cows was free from penicillin by 72 hr. post-infusion. The migration of antibiotics to uninfused quarters was studied and reported in Table 1.

Although many untreated quarters exhibited positive results, caution should be employed in interpreting the observations. The zones of inhibition of the milk from untreated quarters were quite small and questionable in some cases. One could assume and project that an equivalent amount of antibiotic-free milk added to the volume of milk contained from the positive quarter(s) would result in negative readings, when the sensitivity of the test used in this experiment (.05 unit penicillin/milliliter of milk) is considered. This dilution would not effect proper bacterial action for making fermented dairy products.

The fact that there was a greater penicillin migration in aqueous solution than in oil vehicles is also reported by Hueber *et al.* (5) and Ullberg *et al.* (9, 10). These workers stated that aqueous infusions are superior to ointments or oil suspensions in therapy, due to their greater spreading and penetration within the udder.

The numbers are small, but it is noteworthy that the milk of two of three mastitic cows showed no transfer of penicillin from treated to untreated quarters. The milk from untreated quarters of one mastitic cow exhibited the presence of penicillin for 12 hr. post-treatment, with both aqueous and oil suspension. Cases reported by other authors of transfer of antibiotics from treated to untreated quarters involved apparently normal cows, and severe cases of mastitis could give different results.

These data indicate that penicillin is not necessarily localized following intramammary infusion.

TABLE 1

Presence of penicillin in untreated quarters after infusion[a]

Cow	1. Oil suspension	2. Aqueous solution
1624	None[b]	RF[c] and LF[c] for 12 hr.
1452	None	LF for 12 hr.
1650[d]	RF for 12 hr.	RF for 12 hr.
1611[e]	RF for 12 hr., LF at 24 hr.	RF for 12 hr.
1620	None	RF for 12 hr.
1608	None	RF for 12 hr.
1249[e]	None	None
1487	None	RF and LF for 12 hr.
1550	LF at 24 hr.	RF for 12 hr.
1652[e]	None	None

[a] Infusion rate, 100,000 units procaine penicillin G in the right rear quarter.
[b] No detectable penicillin in the untreated quarters based on the Arret and Kirshbaum test (3).
[c] Right front and left front quarter, respectively.
[d] Right rear quarter reinfused 1 wk. later and the right front quarter was again positive for 12 hr.
[e] Cows infected with mastitis.

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REFERENCES

(1) ANONYMOUS. Antibiotics and Chemotherapeutics in Milk. Animal Disease Trends, 8(1): 3. 1960.
(2) ANONYMOUS. Antibiotics Residues in Milk. Animal Disease Trends, 8(3): 1. 1960.
(3) ARRET, B., AND KIRSHBAUM, A. A Rapid Disc Assay Method for Detecting Penicillin in Milk. J. Milk and Food Technol., 22: 329. 1959.
(4) HOLLISTER, C. J., HUEBNER, R. A., BOUCHER, W. B., AND DEMOTT, T. Parenteral Benzathine Penicillin V in Bovine Mastitis. Am. J. Vet. Research, 20: 287. 1959.
(5) HUEBER, W. G., LOFGRIN, C. E., REYNOLDS, W., AND LUTHER, H. G. Vehicles for Intramammary Administration. Vet. Med., 45: 395. 1950.
(6) PLASTRIDGE, W. N. Bovine Mastitis: A Review. J. Dairy Sci., 41: 1141. 1958.
(7) RANDALL, W. A., DURBIN, C. G., WILMER, H., AND COLLINS, J. H. Antibiotics Concentration and Duration in Animal Tissue and Body Fluids. 1. Blood Serum and Milk of Cows. p. 421. Antibiotics Ann., 1953-54, Medical Encyclopedia, Inc., New York. 1954.
(8) SHAHANI, K. M. Visual Detection of Antibiotics in Milk by Means of a Dye. p. 883. Antibiotics Ann., 1958-59, Medical Encyclopedia, Inc., New York. 1959.
(9) ULLBERG, S., HANSSON, E., AND FUNKE, H. Distribution of Penicillin in Mastitic Udders Following Injection—An Autoradiograph Study. Am. J. Vet. Research, 19: 84. 1958.
(10) ULLBERG, S., HANSSON, E., AND FUNKE, H. Distribution of Aqueous Penicillin and Penicillin in Oil in Normal Goat Udders Following Intramammary Injection—An Autoradiograph Study. Am. J. Vet. Research, 19: 135. 1958.

COMPARISON OF RATES OF OXIDATION OF PROPIONATE-2-C^{14} BY LIVER SLICES FROM THE CALF AND MATURE BOVINE

Investigations with the bovine and other ruminants have established (1, 2, 3) that the onset of rumen fermentation is associated with increased blood levels of low molecular weight fatty acids which are available for metabolism. These increases have also been found to be related to various changes in the intermediary metabolism of ruminants (7, 8). Sander *et al.* (9) observed that the administration of sodium propionate or sodium butyrate to young calves caused development of the rumen mucosa. It has been suggested that certain metabolic changes, including the ability to metabolize the low molecular weight fatty acids, may result from the stimulus of their availability after the onset of rumen fermentation. To test this hypothesis, the rates of propionate oxidation by liver slices from nonruminating dairy calves 2 to 3 wk. of age and mature, grade dairy cows were compared in vitro. Propionate oxidation was determined from the amount of $C^{14}O_2$ produced during the metabolism of propionate-2-C^{14} by the tissue slices. Two hundred milligrams of slices prepared from fresh liver samples from each animal were incubated in 2.5 ml. of Krebs-Ringer phosphate buffer, pH 7.0, containing 200 μM of phosphate, 20 μM of $KHCO_3$, and 10 μM (0.5 μc.) of sodium propionate-2-C^{14}. Twenty-five-milliliter Erlenmeyer flasks equipped with center wells containing a pleated piece of filter paper were used for the incubation. Following the addition of the reaction components and slices, the flasks were sealed with serum bottle stoppers to prevent the escape of $C^{14}O_2$. Incubations were carried out for 0, 1, or 2 hr. with shaking at 37° C. At the end of the reaction periods, 10% NaOH was introduced into the center wells by injection through the stoppers with a hypodermic syringe. Sulfuric acid (20 N) was similarly introduced into the incubation mixture to terminate the reaction and release $C^{14}O_2$. The flasks were then shaken for 1 hr. at 60° C. to insure complete absorption of $C^{14}O_2$ by the alkali. After dilution of the center well contents, aliquots were taken for the precipitation of $C^{14}O_3^=$ as $BaC^{14}O_3$, according to the method of Van Slyke *et al.* (10), and counted using a gas flow counter.

Since it had been demonstrated (4, 5, 6) that the major pathway of propionate metabolism in animal tissues involves carboxylation to form methylmalonyl coenzyme A, followed by isomerization to succinyl coenzyme A, an investigation of the ability of liver tissue from both groups of animals to oxidize succinate was conducted using Warburg respirometers. The rates of endogenous respiration and succinate oxidation by liver homogenates from young dairy calves and mature dairy cows were found to be essentially the same. These observations indicate that liver tissue from the two groups of animals possess equal abilities to carry out Krebs cycle reactions and, hence,

the terminal steps in propionate oxidation.

From the data presented in Table 1, it is apparent that liver slices from young dairy calves were capable of oxidizing propionate at comparable rates to liver slices from mature dairy cows. None of the differences between groups of animals were statistically significantly different. It seems likely, therefore, that the ability to oxidize propionate is not an adaptive process but rather a constitutive one in the bovine.

TABLE 1

Propionate-2-C^{14} oxidation by liver slices from young calves and mature cows

Incubation time	No. of animals[a]	Propionate-2-C^{14} oxidized[b]	
		Calves	Mature cows
(hr.)		*(μM)*	
1	5	.125 ± .070	.119 ± .011
2	3	.294 ± .081	.263 ± .115

[a] Determinations conducted in duplicate for each animal.

[b] Average values per 50 mg. of tissue dry weight.

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REFERENCES

(1) BARCROFT, J., MCANALLY, R. A., AND PHILLIPSON, A. T. Absorption of Volatile Fatty Acids from the Alimentary Tract of Sheep and Other Animals. J. Exptl. Biol., 20: 120. 1944.

(2) CARROLL, E. J., AND HUNGATE, R. E. The Magnitude of the Microbial Fermentation in the Bovine Rumen. Appl. Microbiol., 2: 205. 1954.

(3) ELSDEN, S. R., AND PHILLIPSON, A. T. Ruminant Digestion. Ann. Rev. Biochem., 17: 705. 1948.

(4) FLAVIN, M., AND OCHOA, S. Metabolism of Propionic Acid in Animal Tissues. I. Enzymatic Conversion of Propionate to Succinate. J. Biol. Chem., 229: 965. 1957.

(5) LARDY, H. A., AND ADLER, J. Synthesis of Succinate from Propionate and Bicarbonate by Soluble Enzymes from Liver Mitochondria. J. Biol. Chem., 219: 933. 1956.

(6) ORBER, V., LIPSON, N., SAKAMI, W., AND WOOD, H. G. Conversion of Propionate to Liver Glycogen in the Intact Rat, Studied with Isotopic Propionate. J. Biol. Chem., 183: 531. 1950.

(7) MCCANDLESS, E. L., AND DYE, J. A. Effects of Alloxan Administration in the Calf. Am. J. Physiol., 156: 355. 1949.

(8) MCCANDLESS, E. L., AND DYE, J. A. Physiological Changes in Intermediary Metabolism of Various Species of Ruminants Incident to Functional Development of Rumen. Am. J. Physiol., 162: 434. 1950.

(9) SANDER, E. G., WARNER, R. G., HARRISON, H. N., AND LOOSLI, J. K. The Stimulatory Effect of Sodium Butyrate and Sodium Propionate on the Development of Rumen Mucosa in the Young Calf. J. Dairy Sci., 42: 1. 1959.

(10) VAN SLYKE, D. D., STEELE, R., AND PLAZIN, J. Determination of Total Carbon and Its Radioactivity. J. Biol. Chem., 192: 769. 1951.

INTERPRETIVE SUMMARIES OF PAPERS

LABORATORY STUDIES ON HIGH TEMPERATURE–SHORT TIME STERILIZED EVAPORATED MILK. I. EASILY CONSTRUCTED ECCENTRIC FALLING BALL TYPE BOMB MICROVISCOMETERS SUITABLE FOR CONTAINING BIOLOGICAL MATERIALS DURING STERILIZATION AND STORAGE

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A microviscometric technique has been developed for the purpose of facilitating laboratory studies on high temperature–short time sterilized evaporated milk. Hitherto, such studies have been limited to investigations on a pilot plant or plant scale. The technique is based on th eemployment of a simple eccentric falling ball type bomb microviscometer. This instrument lends itself to easy construction in large numbers and provides a chamber for containing milk during high temperature–short time sterilization. Viscosity measurements may be made on a single sample, with minimum disturbance of the sample, before and after sterilization and during storage. Thus, the changes in rheological properties of the sample may be followed up to the point at which coagulation or gelation becomes evident. Because the technique is one which may have a broad biological application, the construction and operation of the microviscometer is described in a general way, disregarding for the moment the important specific application to studies on high temperature–short time sterilized evaporated milk.

FORMATION OF A FAT PROTEIN COMPLEX IN MILK BY HOMOGENIZATION

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At the usual homogenizing pressures of 2,000-2,500 p.s.i. the fat phase of milk is finely dispersed and does not readily rise in the skimmilk medium. However, the fat can be almost 100% recovered by flotation if the centrifugal force is sufficiently increased. In the production of foam-dried whole milk powder, milk is concentrated to 50% T.S. and homogenized at 4,000 p.s.i. It was observed that the sediment obtained by centrifuging a reconstituted sample of this milk contained a considerable amount of fat. Since the density of milk fat is less than that of the skimmilk in which it is dispersed, the sedimenting of milk fat could only mean that it was tightly associated with some milk component of much greater density.

Experiment showed that fat-protein complexes which sediment increased

in amount with increasing homogenizing pressure; with increasing fat content; with increase in concentration above 31% T.S.; and with increasing calcium ion concentration up to a limit of 10 m*M* added calcium.

Analysis of the protein associated with the sedimenting fat shows that it is casein. Experiments to determine the manner in which the fat-protein complex is formed indicate that conditions existing at the homogenizer valve at the instant of homogenizing are responsible for the formation of the fat-protein complex.

THE SOLUBILITY IN AQUEOUS UREA SOLUTIONS OF THE MICELLAR CASEINATES OF MILK AND MILK PRODUCTS SUBJECTED TO VARIOUS STERILIZING HEAT TREATMENTS

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Heat sterilization produces a number of undesirable organoleptic and physical changes in milk products, some of the most important of these being due to the heat modification of the milk proteins. Much work has been done on the changes produced in soluble whey proteins but little on the casein micellar fraction.

Because of a possible correlation between gelling in concentrated heated dairy products, and heat effects on the casein micelles, work was conducted on the solubility in 3.3 *M* aqueous urea solutions of the dry caseinates removed by centrifugation from milks with different heat treatments.

The results show decreased solubility of the caseinates, with increased heat treatment, presence of fat, and increased solids content. High-temperature—short-time methods of sterilization generally produce less change than does the conventional method, and the presence of added citrate and phosphate ions increases the solubility of the high-heat-treated caseinates.

An electrophoretically unique heat-produced protein component concentrates in the urea-insoluble fraction and probably contributes most of the tendency of the casein fraction to become insoluble in aqueous urea with heat treatment.

ANALYSIS FOR CITRIC ACID IN PRESENCE OF CASEIN

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Further details regarding analysis of citric acid in the presence of casein are reported, using a method previously published in the JOURNAL OF DAIRY SCIENCE (December, 1958). The slight cloudiness that occurred in analysis of

normal milk introduced a maximum 2.5% error in the determination; however, with redissolved caseins, the error could be as high as 175%. Casein turbidity could be prevented by adding alkali to the test (i.e., pH above 12.3).

Acid casein required seven treatments in fresh washing solution (pH 4.5) to liberate trapped citrate, lactose, and calcium. Using a thoroughly washed casein, adsorption of citric acid (e.g., binding at the polar groups on the surface of casein particles) was shown to occur. This adsorption accounts for the lower citrate concentration measured in acid sera, after removal of the casein.

COAGULATION OF MILK FOR CHEESE MAKING BY ESTER HYDROLYSIS

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The development of acidity in milk with lactic starter culture is the source of many problems in the manufacture of cheese, and restricts the conditions that may be used. These difficulties might be avoided if the milk could be acidified in some other manner. The direct addition of acid to the milk is undesirable, because it causes local precipitation of the casein, and the stirring necessary to mix the acid with the milk causes the formation of a precipitate rather than a smooth coagulum that is needed in Cottage Cheese manufacture.

One solution to the problem is to add to the milk a neutral compound which will be converted to an acid after it is dissolved in the milk. A number of possible compounds were tested and D-glucono-delta-lactone and meso-lactide were found to give excellent results. These compounds are cyclic esters which slowly hydrolyze in the presence of water to form gluconic acid and lactyl-lactic acid, respectively.

These compounds were used to make Cottage Cheese on a laboratory and pilot plant scale with fluid skimmilk and reconstituted nonfat dry milk solids. The cheese prepared had a pleasant, bland flavor. For a more flavorful cheese, starter distillate could be added or the creaming mixture could be inoculated with a suitable lactic culture.

The time required to coagulate the milk could be reduced considerably over that required for lactic starter cultures. In general, the higher the temperature the more rapidly the acid developed. Lactide also hydrolyzes faster than glucono-delta-lactone. However, the temperature can not be raised above about 37° C., for curd obtained at higher temperatures lost whey and matted so quickly that it could not be handled by the usual procedures. A small amount of rennet also reduced the time required to coagulate the milk.

The present process may have some commercial utility. However, it does not appear that the savings in time, labor, equipment, and culture will entirely make up for the cost of the acidogen. The process is not affected by antibiotic and bacteriophage, and it makes possible the manufacture of Cottage Cheese in remote areas from dry ingredients. The use of acidogens gives a more repro-

ducible production of acid than starter cultures, and this makes the present process more adaptable to mechanized cheese making. This technique also shows much promise in studying the effect of various factors on the ripening of cheese.

THE EFFECT OF CHANGING THE FAT AND NONFAT SOLIDS IN MILK

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When this study was begun, a large surplus of nonfat solids (NFS) was available. Movement of surplus NFS into human food channels by standardizing fluid milk to definite levels of NFS and fat seemed to be a reasonable solution to the problem, as well as resulting in milks with more uniform nutritive value.

To test consumer acceptability, milks standardized to 2, 3, and 4% fat and 12, 13, 14, and 15% total solids (TS) were tasted by flavor panels of six experienced milk judges and 38 unexperienced judges. The flavor testing resulted in the selection of samples with compositions of 2-11, 3-11, 2-12, and 3-12, where the first number represents per cent fat and the second NFS. These four samples were not readily distinguishable from each other flavorwise, and were preferred to those containing 4% fat.

The samples containing 12% TS and the regular University homogenized milk tasted flat by comparison with the four samples.

In the TS range of 13, 14, and 15% it appears possible to substitute 1% NFS for 1% fat without affecting the flavor. Standardizing milks to definite levels of both fat and NFS makes possible, (1) the standardizing of the nutritive value of unit volumes of milk, (2) removing NFS from and preventing NFS surpluses, and (3) improving flavor acceptability.

PREDOMINANT BACTERIA IN THE RUMEN OF CATTLE ON BLOAT-PROVOKING LADINO CLOVER PASTURE

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Bloat is responsible for considerable economic losses in areas of the world where ruminants are grazed on pastures containing high proportions of legumes. It is now generally believed that entrapment of ruminal gases in a stable foam

which can not be eliminated is the main cause of bloat. However, little is known of the factors involved in the production of stable foam. Various observations have suggested that the metabolic activities of ruminal bacteria are intimately involved in the etiology of bloat.

In recent years a considerable amount of information has been obtained on methods of culturing and for the identification of bacterial groups functional in the rumen.

The present study was undertaken to determine if differences could be found in bacterial groups cultured from the rumen of three bloating and three nonbloating steers fed on Ladino clover pasture. Studies on the bloaters were made before and after they were treated orally with 50 mg. of procaine penicillin to prevent bloat.

There were no significant differences between animal groups in pH of ruminal contents, counts of cellulolytic bacteria, total anaerobic bacterial counts, or the occurrence of 11 presumptively identified bacterial groups. Counts of facultatively anaerobic streptococci were significantly depressed after penicillin treatment, but the numbers present were too small to make it seem possible that they were involved in the production of bloat.

The results indicate that the occurrence of bloat in animals consuming Ladino clover is not related to large differences in the numbers or predominant species of ruminal microorganisms cultured 4 hr. after feeding.

The microflora of these Ladino clover–fed steers differed somewhat from that usually found in animals fed roughage rations in that total anaerobic counts were high and the proportions of cellulolytic bacteria were very low. Three species of bacteria were present in much higher proportions of the total flora than are usual. Whether any of these peculiarities contribute to bloat is speculative, since the nonbloating as well as the bloating animals had them. It does seem probable that a very active flora suggested by the high cultural count is of significance. It is also possible that the high percentage of gas-producing species cultured is of significance.

A COMPARISON OF THE ALL-GLASS, SEMIPERMEABLE MEMBRANE, AND CONTINUOUS FLOW TYPES OF APPARATUS FOR IN VITRO RUMEN FERMENTATIONS

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Several different types of apparatus have been suggested for in vitro rumen fermentation studies. The all-glass type of apparatus has been used for a number of years in this laboratory; however, the effect of end-product accumulation upon the bacteria had not been studied in great detail. Because various investigators had obtained slightly different results with other types of in vitro rumen fermentation apparatus, it was thought that a comparison of these apparatuses would be of interest for continued work. The all-glass, the semipermeable membrane, and a semipermeable membrane suspended in a continuous

flow of mineral mixture were the three types of apparatus chosen for this comparison study.

Using cellulose digestion, volatile fatty acid production and ammonia nitrogen production (for hay samples only) as criteria, no major differences were found between the different types of apparatus. In addition, microscopic examination revealed little difference between the types of apparatus as far as propagation of the various bacteria was concerned.

The results of this study indicate that the accumulation of end products is without marked effect on the criteria examined. On this basis, the authors would recommend the all-glass apparatus, because of its simplicity.

A THEORETICAL RUMEN FERMENTATION BALANCE

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There is a large amount of information available concerning the importance of rumen microorganisms in the digestion of foodstuffs by ruminants. The dietary carbohydrates are converted by these microorganisms, by a fermentation process, into short-chain volatile fatty acids, methane, and carbon dioxide. The short-chain volatile fatty acids produced in the rumen are absorbed by the blood and are utilized in the metabolism of the tissues of the ruminant. Methane and carbon dioxide are waste products of the fermentation.

It would be useful to be able to describe the rumen fermentation in quantitative terms. The amounts of short-chain volatile fatty acids, methane, and carbon dioxide formed from a given amount of ingested carbohydrate are certainly important factors in determining the efficiency of a particular feeding situation. Unfortunately, these quantitative aspects of the rumen fermentation are difficult to measure experimentally. It is the purpose of this report to attempt to approximate these quantitative relationships by the use of a theoretical chemical equation which represents the fermentation occurring in the rumen.

The method of calculating a theoretical equation for a given distribution of volatile fatty acids in rumen fluid is described. For a molar distribution of 65 acetate : 20 propionate : 15 butyrate, the equation is:

$$57.5\ (C_6H_{12}O_6) \rightarrow 65 \text{ acetate} + 20 \text{ propionate} + 15 \text{ butyrate} + 60\ CO_2 + 35\ CH_4 + 25\ H_2O.$$

Some experimental values are available which can be compared with the predictions of the equation, such as the amount of methane produced per gram of digestible carbohydrate fed, the amount of volatile acids produced per gram of cellulose digested, and total methane production per day.

Application of the balance is made to amplify the interpretation of experimental data concerned with variations in the molar distribution of fatty acids on various dietary regimes. It is proposed that the fermentation balance is useful for interpreting certain quantitative aspects of ruminant nutrition in terms of the quantitative aspects of the rumen microbial fermentation.

SOME EFFECTS OF A SUPPLEMENTARY CELLULASE PREPARATION ON FEED UTILIZATION BY RUMINANTS

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Cellulose, the chief structural material of plants utilized by cattle and sheep, is broken down by cellulase enzymes produced by the rumen microorganisms. The dependence of ruminants on symbiotic microorganisms for roughage utilization suggested that addition of a supplementary cellulase to the rumen might be beneficial either by increasing the rate or the completeness of cellulose breakdown.

Enzyme 19AP, a fungal cellulase preparation, when added to fresh rumen fluid greatly increased cellulase activity as measured by the change in viscosity of a cellulose-derivative solution.

Thirty dairy calves were used to test the effect of Enzyme 19AP on feed utilization in a growth trial. One calf of each group of three served as a control and was fed a grain mixture and alfalfa hay or orchard grass hay. The two other calves in each trio were fed the same diet with 1 g. of enzyme via capsule or mixed in the grain mixture. The enzyme did not increase feed utilization as measured by average daily gain.

The effect of Enzyme 19AP on digestibility of roughage diets by sheep was tested. Basal diets of orchard grass hay plus soybean meal and of alfalfa hay were fed in their natural state and autoclaved. The basal and autoclaved-basal diets were fed twice daily, with and without 500-mg. capsules of Enzyme 19AP prior to feeding.

The enzyme did not affect digestibility of the ration constituents. Autoclaving destroyed the cellulase activity associated with the diets but did not affect cellulose digestion. However, dry matter and protein were digested less in the autoclaved diets.

In these trials, the cellulase preparation, Enzyme 19AP, did not increase feed utilization.

CORRECTION FOR HEMOLYSIS IN A PHOTOMETRIC METHOD FOR ASSAYING SULFOBROMOPHTHALEIN IN THE BLOOD OF CATTLE IN TESTS FOR HEPATIC FUNCTION

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The liver is a vital organ of the body having many diverse functions. Of the many tests which have been designed to measure the level of activity of the liver, one is concerned with the rate at which the liver is able to remove a foreign dye, sulfobromophthalein, from the blood stream. For this test it is very necessary that one be able to measure accurately the amount of the dye

in the blood plasma, especially when hemoglobin from the red cells of the blood also is present in the plasma. This paper presents an improved method for the measurement of the quantity of sulfobromophthalein in blood plasma of cattle after the dye has been injected into the blood stream. It should greatly facilitate the rapid and accurate measurement of liver function in cattle.

IMPROVED PHOTOMETRIC METHOD FOR ASSAYING PHENOLSULFONPHTHALEIN IN THE BLOOD OF CATTLE IN TESTS FOR RENAL FUNCTION

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The proper functioning of the kidneys is necessary for animal health. In certain research problems it is necessary to measure the level of activity of the kidneys. This may be done by determining the rate at which a dye such as phenolsulfonphthalein accumulates in the urine after it is injected into the blood stream. In farm animals it is quite difficult and laborious to collect urine quantitatively. Consequently, one test for kidney function involves the measurement of the rate at which the dye phenosulfonphthalein declines in the blood stream after its injection into the blood stream. This paper is concerned with the improvement in the methods for the measurement of the quantity of phenolsulfonphthalein in the blood after its intravenous injection.

INULIN RENAL CLEARANCE IN DAIRY CATTLE

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Inulin is a complex carbohydrate material which, when injected into the blood stream of animals, is eliminated from the body by the kidneys in a characteristic manner. This paper describes a method whereby the determination of the rate at which inulin is removed from the blood stream by the kidneys is used as a measure of kidney function in cattle. One aspect of kidney function, namely, glomerular filtration rate, is measured by this procedure. The application of this method will be of value chiefly to laboratories of veterinary clinical medicine.

EFFICIENCY OF ABSORPTION OF THYROXINE IN VARIOUS FORMS FROM THE GASTROINTESTINAL TRACTS OF LACTATING COWS

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Previous reports in the literature have indicated that cows vary greatly in

their milk production responses to orally administered thyroidal materials. Such differences in response could be due both to differences in the efficiency with which animals absorb thyroidal materials from their digestive tracts and to differences in lactational response to given quantities of absorbed material. The present study was designed to determine if cows differed significantly in their abilities to absorb thyroidal materials from their gastrointestinal tracts, and thus possibly help to explain the basis for differences in lactational responses of cows fed thyroidal materials.

A new physio-chemical procedure was developed for the purpose of determining the efficiency of absorption of thyroxine from the digestive tracts of cows. This procedure was based on the comparative increase in the levels of blood plasma thyroxine (PBI) as caused by thyroxine intravenously administered and thyroidal materials fed to cows. This procedure was judged to be a very satisfactory one.

In 13 lactating cows, the average efficiency of absorption of thyroxine from thyroprotein (containing 1.06% L-thyroxine) was 13.0%, with a range from 7.7 to 22.4%. Differences among animals were highly significant.

In five lactating cows the efficiency of absorption of crystalline L-thyroxine was 11.6% (range 4.5 to 18.4%), whereas that of thyroxine in porcine thyroglobulin (extract of thyroid gland) was 15.9% (range 6.5 to 25.6%). Again, animal differences were highly significant.

The ability of cows to absorb thyroxine from their digestive tracts may be a significant cause of animal variation in their lactational responses to orally administered thyroidal materials.

HERITABILITIES OF SOME TYPE APPRAISAL TRAITS AND THEIR GENETIC AND PHENOTYPIC CORRELATIONS WITH PRODUCTION

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A type appraisal scheme was subjected to an extensive study to evaluate the importance of the various traits measured. If these traits are to be useful in selecting for milk production they must be correlated with production.

Heritability estimates were obtained for 27 type appraisal traits as were their genotypic and phenotypic correlations with production. Heritability estimates for the type traits were quite variable, ranging from 0.33 for depth of barrel to 0.00 for udder shape. The phenotypic correlations of these traits with production were small.

Selection indexes for breeding value of milk production were computed, utilizing seven variables having the highest estimates of heritability and phenotypic correlation with production. It was found that combining type appraisal traits into selection indexes added little to the accuracy of predicting production if production information is available.

EFFECT OF PROMAZINE HYDROCHLORIDE AND CHLORPROMAZINE HYDROCHLORIDE ON THE MOTILITY AND FERTILITY OF BOVINE SEMEN

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The consumption of tranquilizing drugs has increased at a phenomenal rate during the past few years. While their sedative effects are being widely studied, little is known about their effect on reproduction. One report indicates that a tranquilizer, such as chlorpromazine, behaves as a surface-active agent, and interferes with the metabolism and survival of ram spermatozoa. Other reports suggest that this drug may depress cellular metabolism. The latter effect might be useful in prolonging the survival of spermatozoa in vitro. In view of the widespread use of tranquilizers, and the limited information available on their effects on reproductive cells, a study of the effects of promazine hydrochloride and chlorpromazine hydrochloride was undertaken.

These drugs first were added to bull semen in levels ranging from 0 to 2,000 μg. per milliliter of a 20% egg yolk semen extender. The highest levels of both drugs tested were spermicidal, and none of the levels of promazine hydrochloride improved sperm survival. However, sperm survival was prolonged by the replacement of sulfanilamide with 200 μg. per milliliter of chlorpromazine hydrochloride in the CU-16 type of egg yolk extender. The same drug was spermicidal at the 200-μg. level when added to 50% yolk-citrate-sulfanilamide and skimmilk extenders.

The fertility of semen extended with a 20% egg yolk–citrate-glucose-glycine extender containing chlorpromazine gave slightly higher fertility results than a 50% egg yolk–citrate-sulfanilamide control extender. However, when it was compared with the standard sulfanilamide-containing CU-16 and CUE extenders there was no difference in fertility. Therefore, it appears that chlorpromazine hydrochloride, as tested, was not harmful to the fertility of bull semen; neither was the slight improvement in sperm survival with this drug accompanied by any improvement in fertility.

CORRELATION AND RELATIVE PRECISION OF TWO METHODS OF MEASURING THE CHLORIDE CONTENT OF MILK

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A new method of measuring chloride content of milk, based on the potential of a silver electrode, has just been reported. The objectives of this investigation were to compare the relative precision of the new method with that of an accepted method (Mohr), and to establish the relationship of values resulting from each method.

Repeatability of duplicate samples was greater than 0.99 for both methods,

indicating that little or no difference in precision existed and that single determinations by either method were adequate for routine laboratory work.

Single determinations by each method on the same milk sample were highly correlated (0.92), and relationships were linear. An equation for predicting Mohr values from potentiometric values was presented, but since the end point for the Mohr test may vary considerably between laboratories, it should be used on other data with considerable reservation.

COMPARISON OF RATES OF OXIDATION OF PROPIONATE-2-C^{14} BY LIVER SLICES FROM THE CALF AND MATURE BOVINE

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The onset of rumen fermentation is associated with a marked increase in the production of the low molecular weight fatty acids in the rumen. The presence of certain of these acids stimulates the development of the rumen mucosa. The present investigation was conducted to determine whether the ability to metabolize the low molecular weight fatty acids results from the stimulus of their availability after the onset of rumen fermentation. It was observed that liver slices from young dairy calves were capable of oxidizing propionate-2-C^{14} and succinate at rates equal to those of liver slices from mature dairy cows. It appears, therefore, that the ability to oxidize propionate is not an adaptive process but rather a constitutive one.

SYMPOSIUM: MILK SOLIDS-NOT-FAT[1]

INTRODUCTORY REMARKS

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In pioneer days, our ancestors worked hard to perform endless manual chores, and ate heartily to give their bodies strength and energy. During that generation people were lean and hungry. They counted calories to get more meat on their bones. In later generations, those who grew fat from soft living and overeating shortened their life span.

Today we are fat conscious and count calories to keep fat off our bodies. Partly responsible for this complete reversal of attitude is the fact that we do not walk as much or do as much manual work as our forebears did. Labor-saving devices, new techniques, and machinery now provide a considerable amount of the energy once furnished by the human body.

When the Agricultural Research Service listed items of priority for research this year, human nutrition was first and genetics involved in animal breeding and improvement was second. It should be noted that this includes research on solids-not-fat and protein in milk. Those interested in this field have a sound basis for justifying financial support for their research programs.

Some of you may have noted an advertisement in a recent magazine of national prominence. This advertisement guaranteed a cereal that contained fewer calories per bowl than any other brand. This means the company was selling their product on the basis of what it did not contain as far as calories were concerned. We frequently hear statements such as this from housewives: "When I shop I choose the low-calorie foods and beverages." This is a new pattern, and milk salesmen must recognize this to keep milk in its rightful place in the human diet. Thus there should be much more emphasis on the protein and solids-not-fat content of milk. If this is done, the peculiar trend we experience today can be of a great advantage for milk protein constituents so essential in the human diet. These ingredients become of increasing importance in partially restricted food intakes.

It is generally recognized that the dairy production people are just a trifle late in getting their program under way. For example, in Holland where the calorie counters are far less numerous than in America, milk is now solid on the basis of both protein and fat content. They are equipped to test more than 5,000 samples of milk for protein content in one day by a staff of seven persons. The laboratory has a capacity of 15,000 samples a day.

The topics to be presented on the symposium by Dr. B. L. Herrington, Cornell University; Dr. J. E. Legates, North Carolina State College; Dr. S. N. Gaunt, University of Massachusetts; and Dr. R. E. Erb, State College of Washington should provide us with the opportunity to modernize our appreciation of this field. The achievements and background evaluation summarized for each man participating on the symposium will not be repeated. It is of special value to have these papers published in the Journal of Dairy Science.

[1] Papers presented at the symposium on Milk Solids-Not-Fat at the 55th Annual Meeting of the American Dairy Science Association, Utah State University, Logan, June 19-22, 1960.

TESTING METHODS AND MARKETING ASPECTS OF PROTEIN VS. SOLIDS-NOT-FAT

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CHANGING TIMES

Students are sometimes told that changes are natural and inevitable—that we should not consider change as a calamity but as an opportunity. Changes may become calamities for those who oppose them, but they present opportunities for those who look ahead and prepare for them. All this can be illustrated with examples.

The Dairy Industry has experienced many changes. Let us examine one change which is occurring now, and consider what adjustments should be made to profit by the opportunity it offers. What should we do when people put skimmilk instead of cream on the dining table? The per capita consumption of cream has decreased 30% in the last 15 yr. The consumption of skim is increasing rapidly. Everybody does not yet have a chance to buy skimmilk but, in the women's dormitories at Cornell University, the future housewives can choose between half-pint cartons of white milk, chocolate milk, or skimmilk. This was their choice during May of 1960: white milk

67%, chocolate milk 3.9%, skimmilk 29.1%.

Our grandparents prized milk chiefly for its fat content. Milk was converted to cream or butter before it went to market. In those days, skimmilk had no commercial value. It was good only for pigs and chickens. Fat was king of the market place.

This fact was generally accepted that it became part of our language. We are all familiar with such expressions as The cream of the crop, The fat of the land. We know that rich foods are high in fat content and in calories. To skim a book means to select only the best parts and skip the rest. Skimmilk became a term of contempt.

But times have changed. People are on reducing diets. Ice milk is displacing ice cream. The per capita consumption of butter has decreased by half. Skimmilk has become respectable and is on the front row in display cases. It commands a price only a little lower than that of whole milk. In fact, its price per pound of food solids may be higher than that of whole milk. Huge amounts of dried skimmilk are now used for human food. Cottage Cheese is one of our most important products. Even the producers of Golden Guernsey Milk are becoming apologetic. Some of them remove part of the fat, reducing the test to 2%, and sell it as Golden Gold Milk.

Changes are here to stay, at least during the near future, which is of greatest concern to us. We must not ignore these changes; we should study them to see what changes we can make which will be most profitable under these new conditions.

One of the most obvious needs is to change the goal of our breeding program. Why strive to produce more milk fat when the public does not want more fat, and it is unwilling to pay the cost of its production? Let us strive instead to increase production of those milk constituents which the consumer does want.

PAYING FOR MILK

In practice, this means that we must change the basis of payment for milk products. The commercial value of milk products is no longer determined by their fat content. This fact is most clear in the case of bottled skimmilk. It is also clear in the case of Cottage Cheese and milk powder. It is emphasized by the low demand for cream and butter. A realistic basis for payment must recognize the value of the nonfat milk constituents. We need a measurement which can be applied to all dairy products, to skimmilk as well as to cream and whole milk.

Two different plans have been proposed for measuring the value of skimmilk. One would base the price upon total solids content. This seems particularly good when the milk is to be dried, because the yield of milk powder is directly related to solids content. This plan does not seem so good to the cheese maker. He would prefer to pay on the basis of casein content, or protein content, because they are more directly related to yield of cheese.

Payment for protein instead of solids would ignore the lactose content of milk, but lactose is of limited commercial value at present. There is very little market for it. Many cheese factories discard large quantities of lactose in the whey. It may be a liability instead of an asset, because it can create a very serious waste disposal problem. At the present time, there is no good reason to encourage the production of more lactose. We can not use the value of lactose to support an argument favoring payment for total solids over payment for protein. We must look elsewhere for arguments in support of one plan over the other.

Both plans have been used for payment on a limited scale. We might use both methods in the future, but this could cause much confusion and dissatisfaction which would be avoided if we could all agree to use the same method.

Each method of payment, for protein and for total solids, has its own advantages. We should study all aspects of the problem before choosing one method over the other. We should recognize, for example, that the choice of method will influence the relative market values of milk of different breeds, and the herd associations will be concerned about this. We know in general the Guerney milk is richer than Holstein milk, but how much richer? That is an important question when you are buying or selling milk.

According to the data of Overman *et al.* (15), Guernsey milk is 18% richer in terms of protein, but only 8% richer in terms of total solids. If we sold skimmilk powder according to protein content, Guernsey powder would be more valuable by 9%. These are the facts of life. Should we demand a higher price for a product containing 9% more protein, or should we sell both at the same price per pound? What does the farmer do when he buys feeds of different protein content?

We might inquire what effect each plan would have upon the success of our breeding programs. It is obvious, of course, that in one case we would select for increased solids and in the other for increased protein. I suspect that the second might yield quicker results, because the goal would be more sharply defined and progress could be more accurately measured. The results of analyses for total solids might confuse the breeder, because a significant increase in one component of milk could be masked by a decrease in another. In effect, under a solids-not-fat program, the breeder would be working partially in the dark and progress might be slower than if he were selecting for protein alone.

EMPHASIZE PROTEIN

We all have one big goal, to sell as much milk as posible to all consumers. May their numbers increase and their appetites, also. Is it possible that the method of payment may affect the sales of milk? At present, we think and talk in terms of fat content, but fat does not have the sales appeal that it used to have. Let's stop talking about fat and talk about protein. The word protein has magical properties today. Home economists, nutrition experts, and ads in our newspapers and magazines all stress the importance of protein in the diet. Let us take advantage of all this free advertising. The meat packing industry has promoted the sale of meat by telling of the high quality of its protein. Cereal manufacturers are promoting special high-protein cereals. Bakers sell high-protein bread. Let us take advantage of this situation and stress the fact that dairy products are excellent sources of high quality protein. We can emphasize this fact by pricing according to protein content. At the same time we can take advantage of the fact that the customer is willing to pay a higher price for protein than for any other class of foodstuff. If you compare the price of steak with the price of bread and potatoes on one hand, or with lard and margarine on the other, you will see the advantages of classifying dairy products as protein foods in the mind of the consumer.

Many people are buying skimmilk for table use. They believe it is rich in vitamins, minerals, and proteins. We could tell them it is rich in nonfat dry milk solids, but I doubt if that would be a good sales argument. Let's not mention nonfat dry milk solids at all. No salesman can get enthusiastic about such a mouthful of syllables. No housewife would stop to listen. No radio announcer can speak the words nonfat dry milk solids with the same caressing accents he uses to say protein.

ECONOMICS OF ANALYSIS

Before deciding whether to pay on the basis of solids or protein, we should investigate the practical problem of analysis from the standpoint of cost, speed, and accuracy. First, we should recognize that we can not abandon the fat test as a factor in payment. A new test will add to present costs, not reduce them, but the cost of testing would be small and should have no effect upon the retail price of milk.

Secondly, we do have a variety of methods available for both tests. They differ in speed and accuracy, in the initial cost of equipment, and in the cost of operation, but it is possible to use either method of testing. I have heard a few reports of manufacturing plants in this country which used solids as a basis for payment. It is well known that, in Holland, payments have been based on protein tests since 1957 (16). One central laboratory is able to make 10,000 protein tests per day (18).

I would like to review some of the methods which might be used for testing, and point out some of the problems involved. These problems need not be insurmountable, but they do deserve attention.

PROBLEMS OF ANALYSIS

Drying, with or without vacuum, is the most obvious way to determine total solids. It seems simple, but is is well known that different laboratories have difficulty in checking each other. The time and temperature, the size of sample, and size of dish must be standardized if reproducible results are to be obtained. The technique of weighing is important because evaporation from the dishes, and creaming in the pipette, can cause errors.

Assuming that the details of technique can be standardized so that reproducible results are obtained, we have a more difficult question to answer. Are these results right? Are they too high or too low? In one sense, the answers are unimportant. If we all agree to buy and sell on the basis of one standard test, it does not matter whether the test is right or wrong, so long as the results are reproducible. If we do not agree upon a single test, if we use two or more different methods, then we must face the question of which one is right.

This may be impossible to determine. It requires a definition of the term total solids. I might define total solids as the sum of the weights of the individual components of milk, but this is not enough. Should the lactose be weighed as anhydrous beta lactose, as hydrated alpha lactose, or as something else? This is an important point, because it can change the solids content of milk by more than 0.2%. Which value is correct?

In practice, it is not easy to determine what we are measuring. Sometimes the lactose may not crystallize during drying. It can remain as a glassy material which gives up most of its water rather quickly (5, 9). Sometimes the lactose does crystallize and it is possible to get either alpha hydrate or beta anhydride. In practice, you are likely to get a mixture. The alpha hydrate crystals are very difficult to dry (5). We must learn how to control the physical state of the lactose before we can determine the solids of milk with accuracy.

There is another source of error in drying methods which is hard to evaluate. When lactose is heated with protein, a reaction takes place between them. The product is colored, it is nearly insoluble, and it weighs less than the starting materials. This loss in weight in a Mojonnier solids determination may lower the solids value by 0.25%. At present, I do not know how to prevent this reaction or how to correct for it. It can be reduced by drying at a lower temperature, but that raises other complications.

There are other methods of determining total solids, but these other methods are calibrated

by a drying test of some kind. They inherit all of the errors of the drying methods and they have some new errors of their own.

For example, many people have tried to calculate solids-not-fat from the lactometer reading and the fat test. Dozens of equations have been developed, giving dozens of different answers (10). Since only one answer can be correct, we must look for sources of error. Some were inherited from the drying test, but the lactometer test has errors of its own. The calibration of the instrument may be in error. The temperature may be wrong. The fat may be liquid when it should be solid, it may be solid when it should be liquid. These factors are under the control of the operator, but some sources of error are not.

For example, surface tension tends to pull the stem of the lactometer down into the milk. This force is variable. In rancid samples the surface tension may be lowered by 10 dynes. This can change the Quevenne reading by 0.3°. When using very sensitive lactometers having a stem of small diameter and a large bulb, variations in surface tension have less effect upon the reading.

Secondly, the lactometer equations assume that the density of the fat and the density of the solids-not-fat are the same in all milks. Unfortunately, this is not true. When lactometer readings are made at 60° F., the density of the fat in the same sample of milk can change as much as 2%, depending upon the time allowed for crystallization of the fat. Modern procedures avoid this by using a higher temperature to insure that the fat will be liquid. If that is done, the density of milk fat at a given temperature is reasonably uniform in different milks. This is not true of the solids-not-fat. In particular, variations in the ratio of protein to lactose cause trouble because the density of lactose is about 20% greater than that of protein (25, 26). What is much more important is this: The difference in density between lactose and water is 80% greater than the difference in density between protein and water. For this reason, variations in the ratio of protein to lactose have relatively great effects on the Quevenne reading. This source of error is particularly serious when tests are made on the milk of individual cows where the variation in ratio may be rather large.

The density of milk may be measured by other instruments, but they are subject to all these errors except those due to surface tension. The development of plastic balls of different density, but with the same thermal expansion as water, is an ingenious solution to the problem of temperature control (7). These balls differ in density by 1° Quevenne. In spite of this, it is reported that solids-not-fat determined with the balls is practically as accurate as values determined with a lactometer (6). We may judge from this that variations in density of the solids are so great that we gain no accuracy by reading fractions of a degree Quevenne. Since 1° Q. represents about 0.25% of solids-not-fat, this may be the limit of accuracy of such measurements.

It has been proposed that the density of milk be determined by the rate of fall of drops of milk through a liquid of known density (3). Such a method might be very rapid, but it introduces new sources of error without eliminating any of the old ones.

It has been proposed that solids might be determined by titration with an oxidizing agent (12). Some are skeptical about the possibilities of this method; I am one of them, but I do reserve the right to change my mind.

Lactometry methods have been studied widely because they offer promise of greater speed than the drying methods. More samples can be tested per man-hour. But this is not necessarily true when large numbers of samples must be tested. A number of efforts have been made to speed up the drying tests. For example, Swedish workers have mechanized the whole drying operation so that one operator can run 700 samples per day (4). This is much faster than any lactometer method, and mechanization should result in greater reproducibility of results. In this respect, the Swedish method is said to be better than the procedure of the Association of Official Agricultural Chemists.

If we choose to determine protein instead of solids-not-fat, we have many methods to choose from. By general agreement, the Kjeldahl method is considered the standard. It is capable of a high degree of accuracy but that accuracy is not always realized. In particular, the complete digestion of casein requires a number of hours of boiling with sulfuric acid and this is sometimes cut short. Furthermore, we should recognize that the Kjeldahl test is really a test for nitrogen, not a test for protein. In the case of milk, about 5% of the nitrogen is present in nonprotein form. Some analysts correct for this but all do not.

The reliability of the Kjeldahl procedure is based upon two facts. First, we can determine nitrogen with accuracy; secondly, each of the major proteins of milk has almost the same nitrogen content. Normal changes in the relative amounts of the different proteins in milk do not seriously affect the accuracy of the Kjeldahl determination.

Because the standard Kjeldahl procedure is slow, people have tried to develop faster modifications of it. For example, the final distillation and titration of ammonia can be replaced by a color test with some loss of accuracy but a considerable saving of time (19).

Of all these quick Kjeldahl methods, the Kofranyi method is today the most important (11). It eliminates the initial digestion with sulfuric acid entirely. The sample is distilled with alkali plus enough barium chloride to prevent foaming. Only a fraction of the nitrogen is recovered but, under carefully standardized

conditions, this fraction is quite reproducible. Many people have compared the Kofranyi procedure with the standard Kjeldahl method and it is considered one of the most reliable of the shorter methods. The Kofranyi method was adopted in Holland in 1957 when they first began to use the protein test as a basis for payment (17).

Many other methods have been proposed to determine protein, ranging from very simple to complex. For example, in the presence of excess ammonia milk becomes viscous, and we can estimate protein from the increase in viscosity of the mixture (1).

We can estimate casein from measurements of refractive index. Two procedures have been proposed. We can separate the casein from the milk and measure the refractive index of the purified casein solution (2) or, we can measure the refractive index of the milk before and after the casein is precipitated (8). We must remove the fat by centrifuging, in order to get distinct readings, but the method has promise. However, the differences in refractive index to be measured are small. A sensitive instrument is required and temperature control is important.

Most proteins absorb ultraviolet light near a wave length of 280 mμ. This fact can be used to measure protein with a spectrophotometer. Of course, quartz cells and optics must be used at this wave length. The absorption is actually due to the ring structures in the amino acids, tyrosin, phenyl alanin, and tryptophane. Unfortunately, the different proteins of milk differ a great deal in composition, their absorbing power for ultraviolet light varies over a 3 to 1 ratio and, for this reason, the method does not seem suitable for the mixed proteins of milk.

It is well known that proteins are stained yellow by treatment with concentrated nitric acid. It has been proposed that this reaction might be used as the basis for a colorimetric test using visible light. However, the yellow color is formed by reaction of nitric acid with the same amino acids which absorb in the ultraviolet. The method has the same disadvantages as the direct measurement of 280 mmμ., and it does not seem suitable for milk.

In alkaline solution, proteins yield a violet color with copper, the so-called Biuret reaction. This has been proposed as a test for protein, but the test can not be applied directly to milk, because lactose interferes. Because the lactose must be removed by dialysis, or the protein must be separated by precipitation, washing, etc., the test is not as quick and simple as we would like.

Protein may be estimated by means of the formaldehyde titration. Many modifications of this procedure have been proposed and many names are associated with these modifications: Sorensen, Walker, Pyne, Steinegger. In its simplest form, the milk is first adjusted to a pH near 8.3. Neutral formaldehyde is added. The mixture becomes acid as the formaldehyde reacts with the amino groups of protein. The number of free amino groups can be estimated by titrating back to the original pH. This titration can be used as a measure of total protein, but the conversion factor depends upon the relative amounts of casein and noncasein protein in the milk. This ratio is not constant. In particular, it varies with the stage of lactation (20). Fairly good results can be obtained when testing mixed milk, though some advise that the conversion factor be adjusted for seasonal changes. The method is much less satisfactory for testing the milk of individual cows because of the much greater variation in ratio of casein to whey protein (22). The method has other disadvantages. It is based upon a small titration between two end points which are not very sharp. The uncertainties in the two end points are rather large in comparison with the total measurement.

The most promising of the new tests are based upon methods proposed almost simultaneously by Schober and Hetzel (21) and by Udy (23). In dilute solution, milk proteins are soluble in acid solution forming polyvalent cations. Some acid dyes can react with these cations to form an insoluble precipitate. The quantity of protein can be estimated from the amount of dye precipitated and this can be measured colorimetrically. Schober and Hetzel used the dye Amido Black 10B (Merck, Germany). Udy used Orange G, which is readily available in this country. More recently, Buffalo Black has been recommended for the determination of milk protein (24).[1]

These methods have attracted much attention. They are new. We may expect improvements in them, but even in their present form the standard deviation from the Kjeldahl is very low, approximately .05%, and this is satisfactory for many purposes. A new laboratory was opened recently in Holland using the Amido Black method (13). They began testing 5,000 samples per day with 12 persons. The laboratory was planned to have a capacity of 15,000 samples per day.

We may expect improvements in these methods. At present, it is necessary to add a rather large excess of dye to insure complete precipitation. This excess makes precise color measurements more difficult. We must measure small differences in color in the presence of a large excess of color. Perhaps we can find dyes forming compounds of greater insolubility, so that a smaller excess of dye may be used.

At present, the dyes on the market are far from pure. In general, we do not know the nature of the impurities, or how they affect the test. Before we can use these methods as a basis

[1] Amido Black 10B and Buffalo Black appear to be trade names for naphthol blue black.

for payment, it will be necessary to have some control of the purity of the dyes, or else assurance that the impurities have no effect upon the results of the test.

If we undertake to guide our breeding programs by means of protein tests, enormous numbers of samples will be involved. So far as possible, this testing must be mechanized to cut down operating costs. The details of this will depend upon the method chosen for testing, but the Technicon instrument developed in this country shows what can be done along the lines of mechanization (14). That instrument can be adapted to many analytical procedures. It is entirely automatic, and it is capable of making from 20 to 60 analyses per hour, depending upon the method.

In closing, I would like to make one suggestion. Before we adopt any test to be used as a basis for payment, we must be sure that it is highly reproducible in different laboratories. It is not enough that the test be reproducible in the same laboratory. I can make the same mistake not once but many times. Others can do as well. To discover all of the pitfalls in a method, it is essential that different laboratories work independently, using the same samples. In my opinion, no conclusions about the reproducibility of a method are valid unless this is done. If such a project is undertaken, I would be glad to share in the work.

REFERENCES

(1) Balwant, Rai Puri, and Harkrishnan, Lal Gupta. VI. Viscosity of Milk on Addition of Ammonia. Indian J. Dairy Sci., 8: 100. 1955.

(2) Brereton, J. G., and Sharp, P. F. Refractometric Determination of Casein in Skim Milk. Ind. Eng. Chem., Anal. Ed., 14: 872. 1942.

(3) Brunner, J. R., Jacobs, G., and Madden, D. M. Adaptation of the Falling-Drop Technique for the Determination of the Specific Gravity of Milk. Michigan Agr. Expt. Sta., Quart. Bull., 42: 232. 1959.

(4) Claesson, Olof. Apparatus and Procedure for Large-Scale Testing of Total Solids in Milk. Kungl. Lantbruks-högskol. Ann., 24: 417. 1958.

(5) Eisses, J. The Determination of Water in Milk Powder by Drying in Vacuum Over Phosphorus Pentoxide. Proc. 15th Intern. Dairy Congr., p. 1583. 1959.

(6) Erb, R. E., Manus, L. J., and Ashworth, U. S. Comparative Accuracy of the Plastic Bead Method of Golding and the Lactometric Method of Watson. J. Dairy Sci., 43: 607. 1960.

(7) Golding, N. S. Plastic Hydrometers for Measuring the Density of Skim and Whole Milk. Proc. 15th Intern. Dairy Congr., p. 1566. 1959.

(8) Hansson, E. The Determination of Casein in Milk with the Refractometer. 14th Intern. Dairy Congr., 3: 143. 1956.

(9) Herrington, B. L. III. The Aqueous Vapor Tension of Alpha Hydrate–Anhydride Systems. J. Dairy Sci., 17: 595. 1934.

(10) Herrmann, Louis F. Indirect Estimates of the Solids-not-fat Content of Milk. USDA—Agricultural Marketing Service—Marketing Research Division. March, 1954.

(11) Kofranyi, E. Eine Schnellmethode zur Ermittlung des Eiweissgehaltes der Kuhmilch. Milchwissenschaft, 5: 51. 1950.

(12) Leggatt, A. G., Dinsmore, D. W., and Sproule, W. H. A Rapid Method for Determination of Solids-not-fat in Milk. Canadian Dairy Ice Cream J., 34: 44, 50. 1955.

(13) Mann, E. J. A New Milk Protein Control Laboratory in the Netherlands. Dairy Ind., 24: 990. 1959.

(14) Müller, Ralph H. Automatic Colorimetric Analyzer Eliminates Need for Many Analytical Procedures. Anal. Chem., 30: 53A, 54A, 56A. 1958.

(15) Overman, O. R., Sanman, F. P., and Wright, K. E. Studies of the Composition of Milk. Illinois Agr. Expt. Sta., Bull. 325. 1929.

(16) R., C. F. Friesland begint met betaling van melk naar eiwitgehalte. Off. Org. K. Ned. Zuivelb, 49: 147. 1957.

(17) R., C. F. Eiwitbepaling. Off. Org. K. Ned. Zuivelb, 49: 909. 1957.

(18) R., J. Eiwitonderzoek op grote schaal in Gelderland-Overijsel. Off. Org. K. Ned. Zuivelb, 50: 766. 1958.

(19) Rao, M. B., and Whitney, R. McL. An Improvement in the Direct Nesslerization Method for the Determination of Total Nitrogen in Stored Sterile Milk. J. Dairy Sci., 43: 563. 1960.

(20) Schober, R., and Fricker, A. Beitrag zur Kenntnis der Formoltitration der Milch. Milchwissenschaft, 9: 83. 1954.

(21) Schober, R., and Hetzel, H. F. Über eine einfache, Kolorimetrische Bestimmung von Milchproteinen. Milchwissenschaft, 11: 123. 1956.

(22) Solberg, P., Syrrist, G., and Steinsholt, K. Formoltitrieringen og dens brukbarhet ved proteinbestemmelse i mjolke fra enkeltkyr. Meldinger Norg. Landbruks-høgskole, 36:(6). 1957.

(23) Udy, D. C. A Rapid Method for Estimating Total Protein in Milk. Nature, 178: 314. 1956.

(24) Vanderzant, C., and Tennison, W. R. A Colorimetric Method for Determining Protein in Milk with Buffalo Black. J. Dairy Sci., 43: 435. 1960.

(25) Waite, R. Further Measurements of Milk Solids Not Fat by a Density Method. Proc. 15th Intern. Dairy Congr., p. 1559. 1959.

(26) Waite, R., and Abbot, J. The Measurement of Milk Solids Not Fat by Density Methads. Proc. 14th Intern. Dairy Congr., 3: 699. 1956.

GENETIC AND ENVIRONMENTAL FACTORS AFFECTING THE SOLIDS-NOT-FAT COMPOSITION OF MILK

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Following a long period when fat has been the major milk constituent, attention has begun to focus on the protein, and even the lactose and mineral content, of milk. In some areas concern has arisen because milk supplies have failed to satisfy minimum standards for legal milk. Remedying these situations has been an expedient, but there also has been a growing conviction that the nutritional properties of milk and its competitive position with other foodstuffs can be enhanced by increasing certain of the solids-not-fat (SNF) constituents.

In appraising the opportunity to improve the quality of milk, the important roles which both the genetic and environmental elements can play must be recognized and understood. Approaches to increase the important constituents in milk by breeding or feeding and managemental regimes must, of necessity, be complementary. In the genetic analysis of milk composition the importance of accounting for general environmental factors is well recognized. Workers have, insofar as possible, attempted to separate the confusing environmental contribution to the total relationships, so as to see more clearly the genetic influences. Present information suggests that there also should be a cognizant awareness of the genetic influences on SNF when the assessment of nongenetic influences is undertaken.

Earlier reviews of factors affecting milk composition have been presented by Johansson and Claesson (21) and by Larson (25) and Tyler (35). As the fat content of milk has received a major share of attention almost since the beginning of this century, the present summary will deal mostly with the SNF constituents of milk. However, information regarding fat percentage will be injected where it can assist in clarifying comparative and interrelated associations.

ENVIRONMENTAL INFLUENCES

Season and climate. The influence of season and climate are difficult to separate from nutritional factors. In addition, studies involving herd milks have suffered from the confusing impact of stage of lactation when seasonal calving was practiced. Studies both in the United States (11, 32, 40) and in Europe (4, 18, 38) have shown that the SNF content in June and July are rather consistently lower than in other months of the year. The lactose content generally was highest when the protein content was lowest, and vice versa. Nonetheless, when the average composition is compiled for a cow's entire lactation, each lactation extends over the greater part of the year and season of calving differences are usually small (30).

Some work has definitely pointed out that SNF can be lowered by high environmental temperatures. In controlled climatic chambers, Cobble and Herman (10) found that SNF content decreased as the temperature rose above 90° F. The chloride content increased and the lactose content decreased. Lowering environmental temperatures from 50 to 5° F. produced an increase in fat and SNF, but there was no appreciable change in chloride or lactose content. Presumably, environmental temperatures from 30 to 70° F. do not appreciably influence the composition of milk. Likewise, variations in humidity do not appear to influence milk composition when the temperatures are below 75° F.

State of lactation becomes most important when the influence of certain factors on SNF are being examined in short-term experiments. The content of total solids, SNF, and protein drop rather precipitously after the first test for the lactation, reaching a low value by the second month in lactation. Most data show a reasonably level picture for SNF during the second to the fifth month of lactation, with a gradual rise being displayed toward the end of the lactation (3, 6, 26, 38, 39). Lactose content is highest at the beginning of the lactation and declines linearly during the remainder of the lactation (38).

Gestation also can make an important contribution to the change in SNF during lactation (17). Bartlett (6) and Wilcox *et al.* (40) found that the content of SNF rose beginning with the fourth or fifth month of gestation. Bailey (3), working with the same herd as Bartlett (6) but at a more recent date, found that SNF showed a tendency to rise by the second and third months of pregnancy. However, the steep ascent of the composition curves began at about the fourth month after successful service. It was particularly evident in the material reported by Wilcox *et al.* (40) that the rise in SNF during the later part of the lactation was practically negligible for those cows which were open.

Age of cow. The decline in SNF percentage with advancing age or lactation number is approximately twice the magnitude of the decline in lactation fat percentage. A summary of five studies (2, 6, 30, 39, 40) has shown declines in SNF ranging from 0.21 to 0.45% during the first 7 yr. of lactation. Politiek (29) reports that the decline in SNF with age in his

data must be ascribed to a decrease in lactose content. This general observation held for the data reported by Robertson *et al.* (30). However, their more detailed analysis indicated that while crude protein did not change much with age, the casein percentage declined during the first seven lactations almost as much as the lactose (30). Hence, there is circumstantial evidence that the whey protein or nonprotein nitrogen fraction increases with advancing lactation number.

The percentage of variance due to age influences for the several milk constituents as found by Robertson *et al.* (30) are as follows: Milk yield, 27%; fat percentage, 5%; S.N.F., 18%; crude protein, 4%; casein, 11%; and lactose 23%. All of the reported age changes must be considered in the light of the selection which has taken place in the populations analyzed. In most cases selections probably were made on the basis of milk yield which, as will be pointed out later, has rather consistently shown small negative phenotypic correlations with milk constituents. Hence, some of the observed age change could have been induced by culling on level of milk yield. The part played by age per se is further confused by the possible influence of udder deterioration through normal usage, and the increasing incidence of mastitis with advancing age.

Feeds and plane of nutrition. Reports of attempts to alter the fat content of milk through feeding are numerous, but current information on the influence of specific feedstuffs on SNF is most limited. There is reasonably abundant evidence to demonstrate that the percentage of SNF is substantially influenced by the energy content of the diet. Feeding rations with 25% less than normal energy requirements resulted in a decrease in SNF, of which most of the decrease was attributable to protein change. In other experiments, feeding only 40% of a normal ration for 6 wk. at the beginning of the lactation resulted in a decrease in the average SNF for the entire lactation (14). Even reducing energy intake by 10 or 20% of normal requirements depressed the lactation average for protein percentage, but no appreciable change resulted in lactose content (14). Several other workers (15, 28) have pointed out that underfeeding may reduce the SNF percentage by as much as 0.30 to 0.40. Generally, the results from underfeeding have been in agreement in showing that the protein content is suppressed somewhat more than is lactose.

Holmes *et al.* (20) have reported that SNF increased rapidly from 8.3 to 8.6% in Ayrshires when the concentrates in the ration were increased from 0 to 4.6 lb. per gallon of milk produced. Burt (9) has also shown that SNF content may be increased by feeding above the normaly recommended British standards. However, feeding excess protein did not appreciably alter the protein content, but the nonprotein nitrogen content was enhanced (33).

As was suggested earlier, some of the seasonal variations in SNF may be traceable to changes in feeding. When cows are allowed fresh pasturage the SNF increases, mainly due to an increase in the protein percentage (29). Rook *et al.* (31) have reported that SNF increased within four days after initiating grazing and the increase reached a peak at six days. An increase in casein constituted the most important change in protein, and the lactose content was not appreciably influenced. Bartlett and Hutton (7) have suggested that the increase in SNF which occurs when cows are turned on young pasture may be due to the estrogenic activity of certain grasses. Folley *et al.* (16) have shown that injected or implanted estrogens increased the total solids content of milk, but their results agree with Browning *et al.* (8), in reporting no significant effect from feeding diethylstilbestrol.

Although the information is not extensive, different sources of carbohydrate feeds do not appear to influence the SNF content (5). Within reasonable limits, changing the relative proportion of concentrates to hay does not markedly alter SNF percentage (5). However, in two trials with 69 cows, Dijkstra (12, 13) has reported that milk yields, SNF, and the protein content of milk were depressed when grass silage in contrast to hay was fed as the sole roughage. Much additional well-planned work is desired on nutritional influences on SNF.

Disease. Mastitis milk generally has been characterized by lowered protein and lactose content, but with a notable increase in chlorides. Waite and Blackburn (37) have also found that SNF and lactose decreased by 0.25 and 0.38%, respectively, as the total leucocyte count increased to 500,000 per milliliter. Casein content declined when the cell counts approached 1,000,000 per milliliter. Milk samples studied did not include those containing clots associated with clinical mastitis. The rise in body temperatures accompanying certain disease conditions is associated with an increase in fat percentage and a decrease in milk yield and SNF content (23).

GENETIC INFLUENCES

Our current approach to the genetic improvement of traits, e.g., the composition of milk, requires a thorough appraisal of the existing genetic and environmental relationships both between and within breeding groups. Breed differences in SNF have long been recognized, but the economic emphasis on fat content and the lack of satisfactory field procedures for determining the composition of SNF deterred the study of variation within breeds.

Breed differences. The summary of breed differences in the composition of milk which Turner (34) prepared has been used widely

TABLE 1
Breed differences in the percentage composition of milk

Breed[a]	Turner (34) Fat	Protein	Lactose	Ash	T.S.	S.N.F.	Armstrong (1) Fat	S.N.F.
A	4.00	3.53	4.67	0.68	12.90	8.90	4.15	8.96
B.S	4.01	3.61	5.04	0.73	13.41	9.40	4.02	9.39
G	4.95	3.91	4.93	0.74	14.61	9.66	4.99	9.32
H	3.40	3.32	4.87	0.68	12.26	8.86	3.49	8.61
J	5.37	3.92	4.93	0.71	14.91	9.54	5.51	9.49
Range	1.97	0.60	0.37	0.06	2.65	0.80	2.02	0.88

[a] A, Ayrshire; B.S, Brown Swiss; G, Guernsey; H, Holstein-Friesian; J, Jersey.

to depict breed averages. These values are based on early information from small numbers of animals and they may not be representative of the breeds, since genetic variation within each breed thus far studied has been sizable. More recently, Armstrong (1) summarized the literature from Canada and the United States bearing on the composition of milk from the dairy breeds. Even this review failed to uncover sufficient samples which had been analyzed for all constituents to provide a satisfactory basis for breed comparisons. However, the information summarized by Armstrong (1) includes some more recent data and the values for fat and SNF percentages are included as a portion of Table 1.

In reviewing the information in Table 1, it is evident that the range of breed variation is limited for both ash and lactose. Fat content varies more than any other constituent, and most of the variation in SNF is accounted for by variation in protein content. Armstrong (1) concluded that over the past 50 yr. increases both in fat and in SNF content have occurred.

Intra-breed genetic variation. The probable response of a trait to selection pressure is a function of the nature and magnitude of its hereditary variation, often incompletely summed up as its heritability, and its genetic and environmental inter-relationships with other traits. For dairy cattle, heritability and genetic and environmental inter-relationships are most conveniently estimated from the daughter-dam regression and by an assessment of the variation between paternal half-sisters. There is a glaring paucity of data which is satisfactory for such genetic analysis of SNF.

Reports (19) based on identical-twin information have pointed out genetic variation in milk composition, but estimates of heritable relationships are not directly comparable to results from daughter-dam and paternal half-sister analyses. Several reports, each based on limited observations, are now available (Table 2). The most complete study is the one reported by Robertson *et al.* (30) from data compiled for 814 Ayrshire cows. It can be noted that the reported estimates of heritability are reasonably high, and agree remarkably well, considering the divergency in the source of material and the paucity of data in many instances. The values reported by Lankamp (24) and Politiek (29) are higher than the others, possibly as a result of some contribution from herd influences.

Evaluation of the potential for changing the milk constituents must also take into account the magnitude of the variation in each constituent within breeding groups. Information is most limited here, but a summary of pertinent phenotypic standard deviations where they have been given is presented in Table 3. Again, within breeds the trend is for fat to be most variable, followed by SNF, protein, and lactose, in that order.

Phenotypic and genetic inter-relationships. A negative phenotypic correlation between lactation milk yield and fat percentage of approximately −0.20 is rather generally accepted. A negative relationship of about the same magnitude appears to exist for the SNF constituents (30), since the phenotypic correlations of SNF, protein, and casein with milk yield range from −0.08 to −0.26. A tendency for high-yielding cows within the breed to produce milk

TABLE 2
Heritability estimates for milk constituents

Breed[a]	Fat	S.N.F.	Protein	Lactose	Source of data
A	0.32	0.53	0.48	0.36	Robertson *et al.* (30)
H & J	0.33	0.35	.....	.....	Johnson (22)
G & H	.....	0.57	.....	.....	Wilcox *et al.* (40)
A	0.52	0.65	.....	.....	O'Connor (27)
....	0.50	0.45	0.45	.....	Von Krosigk *et al.* (36)
H	0.75	0.70	0.75	0.70	Politiek (29)
H	0.72	0.83	0.76	.....	Lankamp (24)

[a] A, Ayrshire; B.S, Brown Swiss; G, Guernsey; H, Holstein-Friesian; J, Jersey.

TABLE 3
Intra-breed standard deviations for milk constituents

Breed	Fat	S.N.F.	Protein	Lactose	Source
Ayrshire	0.35	0.26	0.20	0.17	Robertson *et al.* (30)
Holstein	0.26	0.18	0.17	0.12	Politiek (29)
Holstein	0.36	0.22	.....	.....	Lankamp (24)

which is slightly lower in the various constituents would be expected.

Phenotypic correlations of fat content with SNF and protein were both near 0.40 in data of Robertson *et al.* (30). Johnson (22) reported positive correlations of 0.35 between fat content and SNF, and Politiek (29) found the correlation between fat and protein to be above 0.50. Fat and lactose content appear to be positively correlated with values of 0.11 (30) and 0.16 (29) reported. In summary, the phenotypic correlations between the percentage content of fat, SNF, protein, and casein appear to be positive and of the order of magnitude of 0.35 or above.

The genetic correlations between milk constituents which have been reported by Robertson *et al.* (30) are presented in Table 4. Johnson (22) has also reported positive genetic correlations between SNF and fat percentage, although the data were too few to suggest more than the sign of the relationship. O'Connor (27) reported that the genetic correlation between SNF and fat percentage was 0.40, agreeing closely with the value reported by Robertson *et al.* (30).

The statistical reliability of estimates of the genetic and phenotypic parameters is considerably less than is desirable, and estimates for all breeds are not available. Furthermore, information on herd influences and possible genotype and environmental interactions is most meager. Yet, we can briefly consider the consequences of selecting for SNF or protein content by drawing on the statistics which have been presented. If selections were made on SNF so as to increase the SNF content by 0.30%, about one standard deviation, fat percentage would be expected to increase by approximately 0.17%. The 0.30% increase in SNF would represent an equivalent change of about 0.20% in protein content. If the interrelationships between the percentages of fat, SNF, and protein as determined in future studies are as strong as present information suggests, some increase in fat content will rather consistently be expected as selection pressure is applied to increase SNF or protein. Some decline in milk yield also may be anticipated as the genetic level for SNF is increased, but the expected decline on the basis of current information would not be excessive. Moreover, it should be recognized that the total yield of SNF can be enhanced most rapidly by increasing milk yield, even though the percentage composition of the individual constituents may decline slightly.

SUMMARY

Evidence has been summarized from published studies which bear on the genetic and environmental influences on SNF composition of milk. Reports concerning the influence of season and climate, stage of lactation, and age of cow appear to be consistent enough to point out the general nature of the relationships. There is a pressing need for solid information on the nutritional influences on SNF, both from a fundamental and from a practical standpoint. The reasons for specific nutritional and physiological responses still are largely unexplained.

Breed and sire differences for SNF constituents, broadly suggesting that part of the variation in milk composition is genetic, have been known for some time. Within the past 5 yr. preliminary estimates of genetic parameters, e.g., heritabilities and genetic correlations, have suggested that selection for SNF constituents should be effective. However, more precise information is sorely needed regarding the magnitude of both genetic and environmental parameters, so as to assist in developing breeding plans as soon as the long-range market re-

TABLE 4
Intra-herd phenotypic and genetic correlations from Robertson *et al.* (30)

	Yield	Fat	S.N.F.	Protein	Casein	Lactose	Ash	
Yield		−0.14	−0.18	−0.26	−0.20	0.08	−0.05	
Fat	−0.01		0.40	0.42	0.43	0.11	−0.03	
S.N.F.	−0.02	0.46		0.81	0.87	0.50	0.12	Phenotypic
Protein	0.22	0.48	0.94		0.93	−0.01	0.10	correlations
Casein	0.05	0.56	0.82	0.96		0.17	0.00	
Lactose	−0.16	0.37	0.67	0.41	0.41		−0.28	
Ash	−0.10	−0.42	−0.32	−0.10	−0.24	−0.86		
				Genetic correlations				

quirements for individual SNF constituents are known.

REFERENCES

(1) ARMSTRONG, T. V. Variations in the Gross Composition of Milk as Related to the Breed of Cow: A. Review and Critical Evaluation of Literature of the United States and Canada. J. Dairy Sci., 42: 1. 1959.

(2) BAILEY, G. L. Studies on Variations in the Solids-not-fat Content of Milk. I. Variations in Lactation Yield, Milk-fat Percentage and Solids-not-fat Percentage, that Are Due to the Age of the Cow. J. Dairy Research, 19: 89. 1952.

(3) BAILEY, G. L. Studies on Variations in the Solids-not-fat Content of Milk. II. Variation Due to Stage in Lactation. J. Dairy Research, 19: 102. 1952.

(4) BAILEY, G. L. Studies on Variations in the Solids-not-fat Content of Milk. III. Seasonal Variations. J. Dairy Research, 19: 109. 1952.

(5) BAILEY, G. L. Studies on the Variation in the Solids-not-fat Content of Milk. V. Variations due to Winter Feeding Practice. J. Dairy Research, 19: 169. 1952.

(6) BARTLETT, S. Variations in the Solids-not-fat Content of Milk. I. and II. J. Dairy Research, 5: 113. 1934.

(7) BARTLETT, S., AND HUTTON, J. B. Some Hormone-Induced Changes in Milk Composition. Intern. Dairy Congr., 1: 135. 1959.

(8) BROWNING, C. B., FOUNTAINE, F. C., MARION, G. B., AND ATKESON, F. W. Milk Response from Feeding Diethylstilbestrol to Identical Twin Cows During Complete Lactations. J. Dairy Sci., 40: 1590. 1957.

(9) BURT, A. W. A. The Effect of Variations in Nutrient Intake upon the Yield and Composition of Milk. J. Dairy Research, 24: 283. 1957.

(10) COBBLE, J. W., AND HERMAN, H. A. The Influence of Environmental Temperatures on the Composition of Milk of the Dairy Cow. Missouri Agr. Expt. Sta., Research Bull. 485. 1951.

(11) DAVIS, R. N., HARLAND, F. G., CASTER, A. B., AND KELLNER, R. H. Variation in the Constituents of Milk Under Arizona Conditions. II. Influence of the Month of Lactation in Cows of Different Breeds. J. Dairy Sci., 30: 425. 1947.

(12) DIJKSTRA, N. D. Comparative Experiments on Making and Feeding Grass Silage with Molasses, Wilted Silage, and Barn-Dried Hay. Verslag. Landbouwk. Onderzoek. Minist. Landbouwk., 's Grav., 64: 2. 1958.

(13) DIJKSTRA, N. D. Grass Silage as the Sole Roughage for Dairy Cattle. Verslag. Landbouwk. Onderzoek, Minist. Landbouwk., 's Grav., 65: 14. 1959.

(14) FLUX, D. S., AND PATCHELL, M. R. The Effect of Undernutrition After Calving on the Quantity and Composition of Milk Produced by Dairy Cattle. J. Agr. Sci., 45: 246. 1954.

(15) FLUX, D. S., AND PATCHELL, M. R. The Effect of Short Periods of Undernutrition After Calving on the Establishment of Lactation in Dairy Cows. New Zealand J. Sci. Technol., 38: 689. 1957.

(16) FOLLEY, S. J., WATSON, H. M. S., AND BOTTOMLEY, A. C. Experiments on the Chemical Enrichment of Cow's Milk by the Administration of Diethylstilbestrol and Its Dipropionate. J. Dairy Research, 12: 1. 1941.

(17) GORRIE, C., AND HARVEY, W. R. Some Causes of Variation and Covariation in Fat and Solids-not-fat per Cent of Milk from Holstein Cows. p. 71. Proc. 34th Ann. Meet., Western Div., A.D.S.A. 1953.

(18) GRIFFITH, T. W., AND FEATHERSTONE, J. Variations in the Solids-not-fat Content of Milk. Investigations into the Nature of the Solids-not-fat Problems in the East Midlands. J. Dairy Research, 24: 201. 1957.

(19) HANCOCK, JOHN. Monozygotic Twins in Cattle. Advances in Genetics, Vol. 6. Academic Press, New York. 1954.

(20) HOLMES, W., REID, D., MAC LUSKY, D. S., WAITE, R., AND WATSON, J. H. Winter Feeding of Dairy Cattle. IV. J. Dairy Research, 24: 1. 1957.

(21) JOHANSSON, I., AND CLAESSON, O. Factors Affecting the Composition of Milk. Vol. 3, Chap. 21. Progress in the Physiology of Farm Animals, Butterworths Scientific Publications, London. 1957.

(22) JOHNSON, K. R. Heritability, Genetic and Phenotypic Correlations of Certain Constituents of Cow's Milk. J. Dairy Sci., 40: 723. 1957.

(23) KING, J. L. O. Variations in the Quantity and Composition of Milk Yielded by Diseased Cows While Their Body Temperatures Were Elevated. Vet. Record, 67. 432. 1955.

(24) LANKAMP, H. The Composition of Milk in East Friesian Cattle with Special Reference to Protein and Solids-not-fat. Z. Zücht. Biol., 73: 60. 1959.

(25) LARSON, B. L. Nongenetic Factors Affecting the Production of Nonfat Milk Solids by the Bovine. J. Dairy Sci., 41: 440. 1958.

(26) NICHOLSON, W. S., JR., THOMAS, W. R., WILLARD, H. S., AND BROWN, D. C. Effect of Stage of Lactation on the Solid Components of Milk from Individual Holstein-Friesian Cows. J. Dairy Sci., 41: 232. 1958.

(27) O'CONNOR, L. K. The Inheritance of Milk Quality. Intern. Dairy Congr., 1: 158. 1959.

(28) PATCHELL, M. R. The Influence of Undernutrition of Dairy Cows on the Yield and Milk Composition. New Zealand J. Sci. Technol., 38: 682. 1957.

(29) POLITIEK, R. D. The Influence of Heredity and Environment on the Composition of the Milk of Friesian Cows in the Province of Friesland and the Practical Possibilities of Selection on the Protein Content. Dissertation, Agr. Coll. of Wageningen (Netherlands). 1957.

(30) ROBERTSON, A., WAITE, R., AND WHITE, J. C. D. Variation in the Chemical Composition of Milk with Particular Reference to the Solids-not-fat. II. The Effect of Heredity. J. Dairy Research, 23: 82. 1956.

(31) ROOK, J. A. F., AND ROWLAND, S. J. Effect of Spring Grazing on the Constituents of Solids-not-fat of Milk. Intern. Dairy Congr., 1: 204. 1959.

(32) SPECHT, L. W., BRUNNER, J. R., MADDEN, D. E., AND RALSTON, N. P. The Influence of Several Factors on the Protein and Solids-not-fat Content in the Milk of Jersey Cows. J. Dairy Sci., 39: 1337. 1956.
(33) STEIN, J. F. Some Effects of Feeding Excess Protein to Lactating Dairy Cows. Dissertation, Pennsylvania State University. 1957.
(34) TURNER, C. W. Factors Affecting the Composition of Milk. Missouri Agr. Expt. Sta., Bull. 365. 1936.
(35) TYLER, W. J. Genetic Aspects of Solids-not-fat and Its Components. J. Dairy Sci., 41: 447. 1958.
(36) VON KROSIGK, C. M., YOUNG, J. O., AND RICHARDSON, G. A. Genetic Influences on the Composition of Cow's Milk. J. Dairy Sci., 43: 877. 1960.
(37) WAITE, R., AND BLACKBURN, P. S. The Chemical Composition and the Cell Count of Milk. J. Dairy Research, 24: 328. 1957.
(38) WAITE, R., WHITE, J. C. D., AND ROBERTSON, A. Variations in the Chemical Composition of Milk with Particular Reference to the Solids-not-fat. I. The Effect of Stage of Lactation, Season of Year, and Age of Cow. J. Dairy Research, 23: 65. 1956.
(39) WHITE, M. K., AND DRAKELEY, T. J. The Influence of the Age of the Cow on the Yield and Quality of the Milk. J. Agr. Sci., 17: 420. 1927.
(40) WILCOX, C. J., PFAU, K. O., MATHER, R. E., AND BARTLETT, J. W. Genetic and Environmental Influences upon Solids-not-fat Content of Cow's Milk. J. Dairy Sci., 42: 1132. 1959.

REGIONAL PROJECTS AND AN EXTENSION VIEWPOINT

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A. Regional projects

Interested workers met at the 54th Annual Meeting of the American Dairy Science Association in 1959 at Illinois and appointed a committee[1] on milk solids research work composed of a representative from each of the four regions, Southern, Northeastern, North Central, and Western. The functions of this committee are: (1) to serve as an interim committee until formalization within some framework of the Land Grant College Associations has been accomplished; (2) to promote the formalization of the interregional effort; (3) to have each representative of the committee work with project leaders to implement and coordinate the cooperative effort in his region; (4) to implement and coordinate the cooperative effort nationally; (5) to develop standardized procedures for quantitative determination of milk constituents and collection and processing of data.

Actions taken by the committee include:

(1) The recommendation that the study should include 1,500 cows for each region of each of the five major dairy breeds or 6,000 cows per year from each of the five breeds.

(2) A study of methods for measuring solids-not-fat, namely the Watson Lactometer and the Golding Plastic Bead, compared with the Gravimetric-Mojonnier. The following is a summary of comparison of these three methods at five experiment stations.

Some difficulty was encountered in using the Gravimetric method. This method should be used only by skilled operators according to A.O.A.C. procedures. The Watson Lactometer and the Golding Plastic Bead methods were approved as acceptable methods for this cooperative project, along with the official A.O.A.C. procedure using the Gravimetric-Mojonnier.

(3) A study of methods of measuring pro-

[1] J. E. Legates, W. J. Tyler, R. E. Erb, N. D. Bayley, Secretary, S. N. Gaunt, Chairman.

Summary of means, standard deviations, and correlation for three methods of determining solids-not-fat

Breed	No. observations	Means			Standard deviation			$r_{x_1x_2}$	$r_{x_1x_3}$	$r_{x_2x_3}$
		Gravimetric	Lactometer	Beads	Gravimetric	Lactometer	Beads			
Ayrshire	150	8.92	8.90	8.98	.41	.35	.40	.909	.842	.898
Guernsey	194	9.16	9.15	9.15	.50	.39	.44	.816	.741	.871
Holstein	197	9.27	9.31	9.34	.46	.36	.39	.748	.718	.920
Jersey	233	8.78	8.75	8.73	.49	.37	.39	.711	.685	.914
Brown Swiss	200	9.69	9.71	9.80	.45	.33	.36	.646	.647	.818
Milking Shorthorn	45	8.57	8.51	8.56	.52	.47	.51	.945	.936	.977

x_1 = Gravimetric.
x_2 = Lactometer.
x_3 = Plastic beads.

teins, namely Orange G Dye and Formol Titration. Work at three stations gave correlations between Orange G Dye and Kjeldahl of 0.956, 0.98, and 0.98, and Formol Titration of 0.96, 0.83, and 0.86. Both Orange G Dye and Formol Titration have been approved as acceptable methods for this cooperative research project, along with the official A.O.A.C. Kjeldahl.

(4) An agreement that the data would be pooled only on a lactation basis. The data obtained from the monthly reports would remain at the individual stations. It was also agreed that the accumulation of the lactation data would be carried out by each region according to procedures agreed upon in that region. The pooling and analysis procedures on an inter-regional basis would be worked out at a later date. Minimums regarding data requirements were developed and a form for reporting the information. USDA's Agricultural Research Service is printing these forms for distribution to cooperating stations.

(5) Concentrated efforts toward the establishment of regional research projects. The status is—in the Southern Region this project is being incorporated in a revision of their existing Southern Regional Dairy Cattle Breeding Project. In the North Central Region it will be carried on within the framework of the North Central Regional Dairy Cattle Breeding Project. In the Western Region a regional project has been proposed and a technical committee appointed. In the Northeastern Region a regional project has been proposed, but a technical committee has not been established.

(6) Work toward support of projects. As yet, to our knowledge, no federal funds for this work through regional projects set up or contemplated have been allocated for any of the four regions. However, we are requesting regional support again and wish you would do likewise. Other possible sources of funds have been discussed and are being investigated.

(7) Cooperating in arranging for and holding this symposium on Milk Solids.

B. An extension viewpoint

The dairy industry is at an important crossroads. The road of the past and present has been and is based on a pricing system and, consequently, breeding systems geared to butterfat. The other road, a newer one, is built on the nutritional aspects of milk, particularly the nonfat portion, and is receiving increasing attention. Current trends away from the high fat foods toward high protein diets are well established. Butterfat has lost much of its consumer appeal.

The industry needs to take this new road and with it an appropriate pricing system. At the same time, in order to utilize effectively this new structure and its market opportunities, more basic information is needed. Specifically, knowledge on the control of the production of these solids-not-fat (SNF) components is meager. It is to meet this need that we have launched this nation-wide effort to establish regional projects and to coordinate them.

Industry needs to take a positive approach. It is our feeling that milk should be sold on a positive basis—on its most valuable nutrients, especially protein. The psychology of this in itself, as it filters through the entire industry and the public, could give the dairy industry a big boost.

We believe, to encourage the production of these more desirable components, that the price paid should be the same for the last ounce of SNF or protein as it is for the first. Certainly, it is just as valuable nutritionally. Further, recent research has shown that milk with the SNF content stepped up as much as 10-12% had the highest consumer acceptability. In fact, skimmilk with added SNF, a relatively new product, is being consumed in rapidly increasing quantities. In 1959, sales of fortified skimmilk in 61 markets amounted to 465 million pounds. This represented 2.9% of all the milk beverage sold in these markets, according to the Agricultural Marketing Service of USDA, and was a 23% increase over 1958.

The present price differential for fat percentage has encouraged the production of low-fat milk. This, in turn, has lowered SNF and protein percentages since, on the average, there is a correlation of about 0.4 between fat percentage and SNF and approximately the same between fat and protein. This has created a problem in some markets, with the SNF content lower than minimum state requirements.

If cows were selected and milk sold on an SNF or protein basis, there would be a tendency for fat to go up, too. However, limited information indicates some cows vary from this. How much is one of the things we need to know. It may be that SNF or protein can be increased on many animals without materially increasing butterfat percentage. However, even if fat does go up some with the higher SNF or protein, the fat can be removed or SNF added to the point of maximum desirability for the market and the producer paid for the SNF and protein.

One can make a strong case for testing and selecting cows for protein alone, because of its high nutritional value and the fact that it requires just one test rather than two, as with SNF. Further, the need for high biological protein is world-wide and milk supplies it at low cost. This is important, because the average quart of milk contains enough protein to supply about 45% of the human's daily requirements for protein. Limited work to date indicates that protein varies considerably and that it contributes more to the variation of SNF than lactose. Further, this evidence indicates that these differences are highly heritable and if so, the protein content of milk could be increased by breeding.

Dairy cattle breeders urgently need genetic information on the SNF and protein content of milk, to make intelligent decisions about changing their selection practices to meet the new importance of these constituents and further market changes. The availability of this information could result in widespread changes in the programs of the nation-wide dairy herd improvement associations, the several purebred dairy cattle associations, and the artificial breeding associations.

Product composition, product yield, and processing problems are directly involved with SNF constituents. For example, problems related to yield and properties of Cottage Cheese, cream, evaporated milk, milk powders can be approached on a sounder basis if detailed information, including genetic data, is available on milk constituents.

We are endeavoring to secure these data now in sufficient numbers on a uniform basis to give accurate answers. Since dairy cattle breeding is a slow process, this project needs full support, so that we may have this information very soon to meet the industry needs.

FIELD TESTING FOR SOLIDS-NOT-FAT AND PROTEIN[1]

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Three recent studies (15, 17, 21) show that consumers, when given a preference, select milk containing a higher percentage of solids-not-fat (SNF). This preference for SNF may go as high as 12-13%, even though the same percentage of total solids is maintained with butterfat (21).

One can easily visualize changes in methods of merchandising dairy products. Regardless of these changes, other than water, the basic nutrients in milk, on the average, will be proportionately the same unless altered by shifts to a single breed or by genetic alteration of our cow population. We can also visualize that animal proteins will become deficient in many more areas of the world. Because the dairy cow can convert feeds unsuited for human consumption more efficiently than other animals, we can expect more recognition for milk proteins.

Perhaps butterfat content is emphasized in pricing, because those breeds producing milk high in butterfat generally have higher SNF also, and a financial reward is necessary to maintain the SNF in market milk at reasonable levels. If higher average SNF is desired, selection based on fat percentage is indirect and inefficient for the producer and the processor. The positive relationship between SNF and butterfat percentage is largely a significant statistic because of genetic differences between breeds. When breed effects are removed the correlation between percentage butterfat and SNF is not nearly so impressive. Herds of the same breed and similar average butterfat content vary considerably in average SNF. While this generally balances out in a marketing area because milk from many herds is mixed, the dairyman producing milk containing more SNF in relation to its content of butterfat is at a disadvantage.

Rapid tests for SNF and protein are advantageous in a number of ways: Of immediate importance is the opportunity to more positively focus attention on milk's most valuable asset, the protein. Other uses include selective breeding, pricing of milk based on total composition, rapid determination of composition in certain manufacturing processes, and greater emphasis on protein-lactose ratios as they influence palatability of milk and the manufacture and palatability of processed products. When we arrive at the point in this country where concentrate supplies for livestock production are minimal, we ought to have a dairy cow which produces a greater volume of milk protein in relation to other milk constituents. Essentially, this means proportionately more protein and proportionately less butterfat and lactose from available feed supplies. If this can be achieved by selective breeding, and we have every reason to believe it can, now is the time to determine what we can anticipate from selective breeding. Is it safe to assume that this job requires at least 50 yr., and that by this time we will not be enjoying food surpluses in this country?

We believe that present methods are adequate for mass testing of individual cows and that we can successfully use these tests for the benefit of the dairy industry. The purpose of this paper is to describe our methods and their adaptation to mass testing of individual cows.

METHODS FOR FIELD USE

In an earlier paper during this symposium, methods have been discussed. Any remarks concerning methods, therefore, will be restricted to reasons why one or another is particularly adapted for field use. The first criterion of a suitable method is that it be simple and well within the range of understanding of those doing the work. Simplicity, ease, and rapidity of determination, along with low investment in equipment usually go together. Simple meth-

[1] Scientific Paper No. 1974, Washington Agricultural Experiment Station, Pullman. Projects 1296 and 1378.

ods when used by unskilled technicians are often more accurate under these conditions than so-called laboratory methods which are recommended because of greater accuracy. Suitability of one method over another for field application can not be evaluated realistically by skilled laboratory technicians. In our opinion, far too little thought and time have been devoted to this aspect.

The Babcock test, nationally accepted as a suitable test for milk fat, is sufficiently complicated that its application is often badly abused. Without a constant mechanism for education and checking equipment and operators the method is often highly inaccurate. One needs only to refer to a voluminous literature to realize that biases exist in reading the Babcock test, even between skilled operators (2, 13), and even though all other steps are taken precisely the same. Since the gravimetric assay for SNF also requires strict attention to details and, further, depends on an accurate milk fat determination, this well recognized method also has definite limitations.

Lactometry has been used many years. During its existence lactometry has been repeatedly subjected to about the same criticisms. Fleischmann (7), toward the end of the nineteenth century, made the following statements: "Very considerable variations are to be found both in the specific gravity and in the composition of milk drawn even from the same cow at different times (morning, mid-day, and evening). That there is relation between the specific gravity of milk and its percentage of fat and solids is clear; and it is obvious that these three factors are dependent on one another. It is open to question whether the ratio between these three factors is the same for all kinds of milk, and whether it holds universally true and is practically useful, and can be stated in a definite form." Thus, 64 yr. ago Fleischmann recognized the problems still encountered when specific gravity is used for estimating SNF in milk. Since this early time many formulae for converting specific gravity readings to SNF have been published (8, 9). We now generally recognize that these will vary with the conditions of testing and the population of cows involved.

Solids-not-fat. Some method of estimating SNF by measuring specific gravity of milk appears to be the only procedure simple enough for field use at present. Though there are many types of lactometers, the small lactometer of Watson (10, 14) and the plastic beads of Golding (6) appear the most practical. Compared with gravimetric assay, each has accuracy suitable for testing milk from individual cows. We have not used the small lactometer of Watson. However, we have compared the larger model requiring 8 oz. of milk with the plastic beads of Golding using gravimetric assay as the control measurement for SNF (6). On 300 milk samples from six breeds, the standard deviation from regression was 0.17% SNF for the lactometer and 0.18% SNF for the plastic beads. The linear correlation coefficients were: a) lactometer vs. gravimetric SNF = .95; b) plastic bead vs. gravimetric SNF = .95; and c) lactometer vs. plastic bead SNF = .98 (6). Holstein milk showed the lowest correlation (r = .88) between the gravimetric and bead methods of determining SNF (6). Legates (12) assembled data from five states on comparisons involving the lactometer, plastic beads, and gravimetric methods for SNF. Three of the five states showed good agreement between the gravimetric and the two density methods. These correlations were near those previously published (11, 14). The data from all five states showed high correlations for SNF determined by the lactometer or plastic beads. The combined data showed only slightly greater accuracy for the lactometer method; this result is in agreement with our earlier work (6).

Protein-lactose ratios are different from breed to breed. Since the specific gravity of protein is lower than for lactose, milk above average in protein would tend to be estimated low in SNF by a lactometric procedure. Of course, in the reverse situation SNF would be estimated too high (22). In our study, the SNF would be estimated 0.11% too low when the milk is 1% above average in protein (6). Since individual cows of the different breeds rarely have a range greater than 1% in protein, this does not cause undue concern. Under conditions where protein content also is known, this variable can be included in the conversion formula (6).

Protein. Two methods have been successfully adapted to mass analysis in the Netherlands (18). One is the ammonia distillation method of Kofranyi and the other is dye binding with amido black. Orange G dye has been compared with the Kjeldahl procedure in the United States (1). The evidence is that these procedures have high accuracy, are easily reproduced, and simple to run (1, 16, 18, 20, 21). Our experience has been with dye binding using orange G dye. Compared with macro-Kjeldahl, the standard deviation from regression was 0.078% and the correlation .98 (1). Vanderzant and Brown report no real difference between Kjeldahl and ammonia distillation procedures for determining milk protein (18). These workers have also observed a correlation of −0.965 for protein determined by Kjeldahl and the optical density of residual Buffalo (amido) Black (3). Buffalo Black appears to have an advantage over orange G dye because it ties up protein almost instantaneously, with or without shaking, yielding an insoluble complex that can be separated easily and quickly either by centrifugation or filtration (3, 18, 19).

Present farm procedures. Washington State University has developed an IBM-650 punch

card system for handling DHIA, DHIR, and owner-sampler testing (4, 5). Rather than waste our precious counter space with estimated feed records other than grain, we incorporated protein and SNF into the program. Our herds on DHIA may now, at their option, test their cows for SNF or protein with no additional charges for the added calculations (5).

Golding plastic bead jars are substituted for the usual sample jars and by DHIA supervisors. Milk is tempered in the usual way, and while cooling, a sample is pipetted for the Babcock test. When the milk has cooled to 68° F. the number of plastic beads on the bottom of the jar is recorded. One DHIA association is also testing for protein, using the orange G dye procedure (1). One-ounce plastic bottles with screw caps are sent into the field, each containing 25 ml. of the orange G dye–citric acid solution (1). The DHIA supervisor, using an automatic syringe, samples 1.5 ml. of milk into the test bottles. The milk-dye mixture is stable for at least 2 wk., whether stored frozen, hauled in the back of a car, left sitting exposed to light on a laboratory table, or incubated at 80° F. The DHIA supervisor delivers the samples to our laboratory when in the Pullman, Washington, vicinity. Filtering and colorimetric determination is made by our laboratory technicians. Only preliminary work has been done with various colorimeters. This item of equipment is the most expensive; hence, we are looking for a simple, low-cost instrument that has sufficient sensitivity for a determination accuracy of near 0.1% protein.

The DHIA tester does not convert plastic bead readings to per cent SNF. Instead, he records only the number of sinking beads on the IBM monthly herd report. Our computer makes the conversion and if protein also is known this, too, is used in the conversion formula.

The extra work of determining SNF and sampling for protein almost doubles testing time. We see no reason why the final steps of the protein test can not be done by the DHIA supervisor, provided he has a laboratory. Once the necessary equipment has been assembled and organized, the SNF and protein tests combined require little more time than the Babcock test for butterfat. We have not attempted automation, because none of our associations have a sufficient number of cows to justify the additional expense.

PRELIMINARY FIELD RESULTS

SNF and protein testing of individual cows was started in our state in November, 1959. Slightly over 2,000 cows on central processing are being tested each month for SNF. One association with nearly 800 cows is testing also for protein. An unknown number of cows not on central processing are tested for SNF. We are making no effort to gather data from these herds. The cost of doing this by hand procedures is too great and the chances for transfer errors too high to merit consideration as a data source.

Twelve Holstein herds with 650 total cows have completed 6 mo. of testing. The average SNF was 8.41% (range 8.53 for November to 8.33% for March). The average for protein was 3.05% and for butterfat 3.83%. These values are very near those observed for 917 milk samples from Holstein cows tested in July, 1959 (6). In this latter study, the average SNF was 8.50% and the protein exactly the same, i.e., 3.05%. Butterfat averaged 3.62%.

From November, 1959, to April, 1960, five Jersey herds were tested each month for SNF. They averaged 5.6% fat and 9.54% SNF. Protein was not available on these herds. The average protein was 3.82% for 499 samples of Jersey milk studied during July, 1959 (6). Too few Guernsey herds are currently on SNF testing to justify reporting. However, our earlier data indicate that Guernseys are near 9.0% SNF and 3.40% protein (6).

REFERENCES

(1) Ashworth, U. S., Seals, R., and Erb, R. E. An Improved Procedure for the Determination of Milk Proteins by Dye Binding. J. Dairy Sci., 43: 614. 1960.

(2) Bailey, D. E. Study of Babcock Test for Butterfat in Milk. J. Dairy Sci., 2: 331. 1919.

(3) Brown, M. A. Personal communication. 1960.

(4) Erb, R. E. Testing for Solids-not-fat and Protein. Canadian Dairy and Ice Cream J., 39(4): 40. 1960.

(5) Erb, R. E. Electronic Book Work for DHIA. Western Dairy J., 16: 44. 1960.

(6) Erb, R. E., Manus, L. J., and Ashworth, U. S. Comparative Accuracy of the Plastic Bead Method of Golding and the Lactometer Method of Watson for Routine Determination of Solids-not-fat in Milk. J. Dairy Sci., 43: 607. 1960.

(7) Fleischmann, W. The Book of the Dairy. (Translated by C. M. Aikman and R. P. Wright.) Blackie and Son, Ltd., Glasgow and Dublin. 1896.

(8) Herrmann, L. F. Indirect Estimates of the Solids-not-fat Content of Milk. USDA, AMS, Marketing Research Div., Washington 25, D. C. 1954.

(9) Jenness, R., and Patton, S. Principles of Dairy Chemistry. Chap. 8, p. 218. John Wiley & Sons, Inc., New York, New York. 1959.

(10) Lamb, R. C., McGilliard, L. D., and Brunner, J. R. Accuracy of a Lactometric Field Test for Measuring Solids-not-fat Content of Milk. J. Dairy Sci., 43: 491. 1960.

(11) Lawrence, A. J. Lactometric Determination of Solids-not-fat in Milks of Individual Cows. Australian J. Dairy Technol., 13: 144. 1958.

(12) Legates, J. E. Personal communication. 1960.

(13) LEVOWITZ, D. An Appraisal of the Gerber Test for Milk Fat in Milk and Market Milk Products. J. Milk and Food Technol., 23: 69. 1960.

(14) MADDEN, D. E., AND BRUNNER, J. R. Comparative Accuracy of Small Lactometers for Determining the Total Solids of Individual Cows' Milk. J. Dairy Sci., 41: 783. 1958.

(15) MORRIS, H. A. Why Not Standardize the Solids-not-fat in Milk? Proc. 29th Inst. Dairying, p. 111. Washington State University, Pullman, Washington. 1960.

(16) STONE, W. K., CREMERS, L. F., AND THOMPSON, N. R. A Rapid Method for Determining Milk Protein. J. Dairy Sci., 43: 435. 1960.

(17) STULL, J. W., AND HILLMAN, J. S. The Consumer Acceptance of Milk Beverages as Affected by Fat and Solids-not-fat Content. J. Dairy Sci., 42: 1743. 1959.

(18) VANDERZANT, C., AND BROWN, M. A. Determination of the Protein Content of Milk. Southern Dairy Products J., 66: 60. 1959.

(19) VANDERZANT, C., AND TENNISON, W. R. A Colorimetric Method for Determining Protein in Milk with Buffalo Black. J. Dairy Sci., 43: 435. 1960.

(20) VANDERZANT, C., AND TENNISON, W. R. Estimation of Protein Content of Milk by Dye Binding with Buffalo Black. J. Dairy Sci., 43: 854. 1960.

(21) WAHID-UL-HAMID, SAHIBZADA S., AND MANUS, L. J. Effect of Changing the Fat and Nonfat Solids of Milk. J. Dairy Sci., 43: 1430. 1960.

(22) WAITE, R. Further Measurements of Milk Solids-not-fat by a Density Method. Proc. XVth Intern. Dairy Congr., 3: 1559. 1959.

OUR ASSOCIATION

INVITATION FOR NOMINATIONS FOR VICE-PRESIDENT AND DIRECTORS OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

K. L. TURK
Chairman, Nominating Committee
Department of Animal Husbandry, Cornell University, Ithaca, New York

The By-laws and established policy of our Association specify that the membership be invited each year to suggest names of competent individuals from among the members of A.D.S.A. for the consideration of the Nominating Committee in selecting candidates for the offices of Vice-President and two Directors.

Your Committee feels that to do an effective job of preparing a slate of officers it must have the assistance and full cooperation of the entire membership. You are urged, therefore, to suggest the names of prominent, competent members who are willing to fill these important offices of our Association.

Your Policy Committee believes that members who have served on committees of the Association, or as officers and committee members of the sections, have shown more than ordinary interest in Association work. They have had an opportunity to demonstrate to fellow members their ability and leadership and their willingness to give their time to work of the Association. These experienced workers should be especially well qualified to become candidates for nomination to Association offices of greater responsibility.

Our Vice-President to be elected this year should come from the dairy production field. In making your suggestions please keep in mind that, if elected, your nominee will succeed to the office of President the following year. As you consider suggestions for Vice-President certain desirable qualifications, in addition to personal ability, might be considered, such as: previous service as a Director of A.D.S.A.; experience on the Journal Management Committee or the Editorial Board; work on Association or Section Committees; administrative experience and leadership outside of the Association; and a willingness to devote to the Association the considerable time and energy required by the responsibilities of the offices of Vice-President and President.

The established policy of the Association is that selection of candidates for the offices of Directors shall be chosen to provide equal representation from the production, manufacturing, and extension sections. Also, it is believed that occasionally a man from the commercial field, either in production or manufacturing, should be nominated for an office.

Our present group of Directors includes two from dairy production and two from dairy manufacturing whose terms continue next year. As a result, our nominations for the office of Director should come this year from the Extension Section.

In recommending candidates for the office of Director you might want to consider fellow members who have demonstrated an active and devoted interest in A.D.S.A. affairs. Members who have held section offices, committee chairmanships, or other important Association assignments, will have received an insight into

some of the financial, administrative, and organizational problems of our Association.

All suggestions for officers should reach the Chairman of the Nominating Committee not later than January 1, 1961. The Chairman will assemble the lists and then request a biographical sketch from one of the nominators of each candidate. The Committee then will evaluate carefully the several candidates and will arrive at a final slate of two for each office.

Your Committee urges you to give serious and careful consideration to the selection of new officers of our Association. The future progress of A.D.S.A. depends to a considerable extent upon the leaders elected to our highest offices. We want and need your help in selecting candidates from among the most highly qualified and devoted members.

The membership of the Nomination Committee is as follows: K. L. Turk, Department of Animal Husbandry, Cornell University, Ithaca, New York (Chairman); A. C. Fay, 1245 North Bay Shore Drive, North Miami, Florida; W. M. Roberts, Department of Animal Industry, North Carolina State College, Raleigh, North Carolina; C. L. Cole, Department of Dairy Husbandry, University of Minnesota, St. Paul, Minnesota; and R. E. Burleson, Federal Extension Service, U. S. Department of Agriculture, Washington 25, D. C.

THE INTERPRETIVE SUMMARIES

F. J. Doan, Chairman
Journal Management Committee
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The Interpretive Summaries section of the Journal was introduced with the first issue of Volume 43 as a result of the recommendation of the Committee on Publication Policy, concurred in by the Journal Management Committee and authorized by the Executive Board. The motive was to give the large proportion of the A.D.S.A. membership, who are not engaged in teaching or research, a better appreciation of the research reported in the Journal; its contribution to the pertinent body of knowledge; its possible immediate usefulness; and its implication for the future. It was also believed that this section would make the Journal more attractive to potential members of the Association.

Understandably, there has been considerable criticism of the action. The Editor has taken the brunt of this, most unfairly, inasmuch as the Section was not his creation and actually complicated his already tedious work. On the other hand, many members have been favorably impressed by the Summaries, but have been less vocal than the objectionists.

At the Utah meeting in June, the Executive Board approved the recommendation of the Journal Management Committee that the Interpretive Summaries be published separately, as a quarterly pamphlet, to go to all members and a selected list of dairy periodicals, beginning with Volume 44. The Board also authorized a survey to be made after 12 or 18 months' operation on this basis, to determine the attitude and will of the members relative to continuance of the Summaries. This decision was made after it was found that a separate publication would be no more costly (if cheaper printing methods were employed) than present printing in the Journal. It was also felt that this innovation would remove some of the objections to the present section in the Journal. The first issue of the quarterly will probably appear in April, 1961.

It has been pointed out many times that, in our modern society, the scientist has an obligation to explain and interpret the significance of his field of work and his research to his fellow citizens. It appears logical that, to an even greater extent, the dairy scientist should feel a responsibility for enlightening workers in the industry, particularly fellow members of A.D.S.A., concerning the meaning and implications of his research and its results. One way of accomplishing this is through the Interpretive Summaries. Consequently, they should be most carefully prepared by the author(s) with this objective definitely in view.

Some of the summaries appearing in the Journal have not met the objective as well as they might have, largely because the writers have seemingly failed to keep the intended readers in mind. Obviously, no outline can be followed strictly, because of the great diversity of papers, but in preparing the summaries it is felt that a number of points should be considered:

1. The general object of the study, usually, should be stated or explained and related to present knowledge or past findings.
2. It may or may not be desirable to describe the methods or procedures used. If it is, they should be stated in general terms, unless specifics actually can be made use of by the industry.
3. The significant results or conclusions should be mentioned and an attempt should be made to indicate how these enlarge the available knowledge, substantiate or broaden previous ideas, or how they conflict with them.

4. Any new concepts resulting from the work should be briefly explained.
5. Any immediate usefulness of the findings should be emphasized or the possible future importance pointed out.
6. Phases of the problem needing more study might also be noted.
7. The summary should be as brief and as concise as possible to make it.

Of course, it is granted that some research reports may be so specifically scientific or so highly statistical that they have no real meaning to anyone other than a scientist or statistician. Nevertheless, the author(s) should be able, at least, to explain to a layman what he was attempting to accomplish, why he wanted to do so, and to what degree he succeeded.

No one can prepare, initially, an interpretive summary as well as the writer(s) of a paper. Obviously, he is more familiar with the work, the results, the conclusions to be drawn, and the background of previous knowledge into which the research fits. Afterward, some editing for readability or popularization may be desirable. The author(s) should welcome this if it does not do violence to his statements and if it aids in accomplishing the ultimate object of the summary.

Examples of good summaries are those of Campbell and Phelps (page 286) and of Schmidt (page 291) in the February, 1960, issue of the JOURNAL and those of Day and Lillard (page 691) and of Boyd *et al.* (page 699) in the May, 1960, issue.

The preparation of a good interpretive summary—one which will give the uninitiated a sound idea of the purposes and the accomplishments of a research study—is a challenge and an opportunity and it should be accepted as such. In the end, the whole idea will stand or fall on the quality of the summaries published in the quarterly. The venture can be made a very worth-while part of the Association's program.

The Journal Management Committee hopes that the authors of papers accepted by the JOURNAL will be tolerant of the interpretive summary requirement in the interest of a large segment of the A.D.S.A. membership. It further hopes that they will cooperate with the Editor in carefully writing the summaries and in getting them in on time. Finally, it hopes that the entire membership of the Association will give this whole matter careful thought and be prepared to express a meaningful opinion when the membership survey is made.

DISTINGUISHED SERVICE AWARD OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

H. F. JUDKINS, Secretary-Treasurer
White Plains, New York

For some time the Executive Board has been considering an award for individuals who have rendered distinguished service in the dairy industry, either in production or processing.

A committee consisting of J. Hoffman Erb, The Borden Company, Columbus, Ohio; L. C. Moore, U. S. Department of Agriculture, Beltsville, Maryland; H. B. Henderson, University of Georgia, Athens, and H. F. Judkins, ex-officio member, have formulated rules for this award which were approved by the Executive Board at the 55th Annual Meeting. In order that all members may become familiar with the procedures for this award and make nominations, the essential rules are as follows:

I. Name

Distinguished Service Award of the American Dairy Science Association.

II. Donor

The American Dairy Science Association.

III. Nature of the award

The award consists of an appropriate certificate. Allowance may be provided for travel expenses to the meeting where the award is made.

IV. Purpose of award

To recognize distinguished service to and outstanding achievements in the dairy industry.

V. Recipients

The Distinguished Service Award may be awarded to one person a year, but it may be awarded to not more than three persons in any one year. The award shall be given only when a worthy candidate(s) is available.

As a guide to the areas for which the award may be made, the following are suggested:

1. Leadership and promotion of quality in dairy products.
2. Managerial success in a dairy venture.
3. Development of leadership of people in the industry.
4. Leadership in the organization of state, national or of international dairy organizations.
5. Leadership in developing dairy corporations or cooperatives.
6. Leadership and promotion of legislation of benefit to the industry.
7. Development and dissemination of infor-

mation to the public related to the dairy industry.

8. Promotion of sale of dairy products or dairy cattle.
9. Development of dairy cattle breeds.
10. Promotion of education in the dairy industry.
11. Promotion or development of efficient farm practices related to dairy production.
12. Development of a dairy product or a process which promotes the increased use of milk.
13. Leadership in nutrition education relating to milk and milk products.
14. Development of inventions, equipment, or mechanism procedures in the dairy industry for either the production or manufacturing field.
15. Leadership in promotion and development of special plans affecting the dairy industry, such as marketing dairy products abroad, cow pool, DHIA testing, and artificial insemination.

VI. Eligibility for nomination

1. A nominee may be a member or nonmember of the American Dairy Science Association.
2. May be of either sex.
3. May be active in his field of endeavor or may be retired.
4. Must have made an outstanding contribution to the dairy industry, considered in the broadest meaning of service.

VII. Selection committee

A Distinguished Service Award selection committee shall be set up in the same way as the selection committees are organized for the other American Dairy Science Association awards, six members consisting of five voting and the chairman who has no vote.

VIII. Nomination for the award

1. Any active member in good standing, life member, or honorary member of the American Dairy Science Association may make a nomination, except if such member is an the Selection Committee for the award.
2. A member may nominate no more than one person in any one year for the award.
3. Nominations are to be sent to the Secretary of the Association.
4. Nominations must be in the hands of the Secretary not later than December 15 of the fiscal year in which the award is to be made.
5. Nominations should be accompanied by six copies of complete information on the nominee, as indicated in the nomination blank.
6. In order that no outstanding candidate is overlooked, the Chairman of the award's Selection Committee may contact the dairy department chairmen of the state universities and colleges with curricula in dairy production and dairy manufacturing. He may contact also other society members known to be in a favorable position to evaluate outstanding leadership in the field of the award, publicizing the award and deadline dates for submission of names and supporting data. The Secretary, in addition, may suggest to potential nominators, candidates who may have been overlooked. All nominations, with six copies of supporting data, must be in the hands of the chairman of the Selection Committee by February 1.

IX. Presentation of the award

The Distinguished Service Award shall be presented by the President of the Association as a part of the Awards Program held in conjunction with the Annual Meeting.

X. A copy of the nomination and biographical sketch form to be used by nominators is as follows:

DISTINGUISHED SERVICE AWARD
OF THE
AMERICAN DAIRY SCIENCE ASSOCIATION

(To be furnished in six copies by nominator)

1. Name of nominee .....

2. Date and place of birth .....

3. Present address .....

4. Present position .....

5. Education .....

Date	College attended	Degrees	Where degrees obtained
.....	.....	.....	.....
.....	.....	.....	.....
.....	.....	.....	.....

6. Academic and professional honors .....
.....

7. Positions held

Date	Employer	Type of position
.....	.....	.....
.....	.....	.....
.....	.....	.....
.....	.....	.....

8. List of major activities and accomplishments in the dairy industry .....
.....
.....
.....

9. List of national or community interests and accomplishments .....
.....

Note: Use separate sheet(s) of paper for Items 5 to 9.

Date ..... Signed .....

Address .....

Notice of this award and nomination forms are being distributed with 1961 dues notices. Members do not need to wait for these. Nominations will be welcomed by the Secretary up to December 15, 1960.

CALL FOR PAPERS FOR THE 1961 ANNUAL MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION[1]

G. W. TRIMBERGER
Chairman, Program Committee, A.D.S.A.
Department of Animal Husbandry, Cornell University, Ithaca, New York

The 56th Annual Meeting of the American Dairy Science Association will be held June 12-14 at the University of Wisconsin, Madison. Members who wish to present papers must submit titles and abstracts not later than March 1. This deadline must be met to permit publication of titles with the complete program in the May issue and abstracts in the June issue of the JOURNAL. Last year all but four abstracts arrived on time. If a large number of papers are suggested for presentation, those that arrive late may be assigned to be read by title or rejected entirely.

Abstracts must contain no more than 200 words by actual count. Abstracts longer than 200 words cannot be accepted and will be returned to the author(s) by the Committee. An original (on bond paper) and three copies of each abstract must be typed double-spaced. The original and one copy should be mailed to the Chairman, another copy to the Vice-Chairman, and the fourth to the Secretary of the Section before which the paper is to be presented. Eventually, the original copy will be sent to the Editor for publication in the JOURNAL.

In the preparation of abstracts, the style and abbreviations of the JOURNAL OF DAIRY SCIENCE must be used. Please refer to abstracts in the June, 1960, issue of the JOURNAL for guidance. Only initials of authors should be used, except in unusual cases where it may be necessary to use the complete name. The title of the abstract should indicate clearly the nature of the research. It should not be repeated again in the text. The abstract should include, insofar as possible, the design and major results of the investigation. Only completed research should be reported. Brief, essential statistics will make the data more meaningful.

All members of the Association are entitled to present papers. Participation by members of the industry and by senior members of the Association is particularly encouraged. Attention is called to the Dairy Manufacturing Extension Section program, and members are urged to participate. This is a subsection of the Manufacturing Section for 1961. The competitive presentation of papers by graduate students, initiated in the Production Section in 1959 and in the Manufacturing Section in 1960, will be continued in both sections.

Papers submitted for the Annual Meeting should be confined to research that has not been reported previously. Abstracts of research articles accepted for publication by a scientific journal before the annual meeting are not acceptable, because this constitutes a duplication. The Program Committee favors the general policy that an individual present only one paper and that his name appear as author on no more than two. The Program Committee, together with the Association membership, wishes to stimulate vigorous, sound research and realizes that the above restrictions may penalize some members engaged in full-time research. Therefore, the Committee has liberalized this policy to the extent that a member can exceed these limits, but only if he or his department rates the abstracts in order of preference for oral presentation. If the total number of papers submitted by the membership is too great to include in the program, the Committee will assign some papers to be read by title only. In this event, consideration will be given to quality of abstract and research reported therein, number of abstracts per author and department, and importance of the research reported.

The Program Committee encourages continued improvement in quality of papers and in oral presentation. Careful design of experiments and proper interpretation of results are necessary prerequisites. Strict compliance with the instructions for preparation of abstracts will simplify the task of the Program Committee, and will improve the program. Careful editing of abstracts before submission is essential. In the past too many abstracts have been lacking in this respect.

All abstracts must be submitted on regular 8½ by 11-in. paper. The following form, with no capitals for the title, is correct and is in accordance with the form used in 1960:

> Utilization of carbohydrates posterior to the rumen-reticulum of the bovine. J. T. Huber and N. L. Jacobson, Iowa State University.

If the author lists an address for an experiment station other than the University, or a USDA research branch, or a commercial company, then the complete address should be provided, as presented in the following example from the 1960 program:

> A study of dye reduction methods as platform tests for the detection of antibiotics. Burdet

[1] The procedures for preparing abstracts will apply also to abstracts submitted for the Eastern, Southern and Western Divisional Meetings of the American Dairy Science Association. Editor-in-Chief.

Heinemann, Producers Creamery Co., Springfield, Missouri.

Preparation of mimeographed copies of pertinent data is desirable. At least 250 copies should be available for distribution. This can be supplemented by slides for projection on screens, provided the author can adhere to the assigned time of 12 to 14 min. for presentation of each paper.

Names and addresses of officers of sections to whom titles and abstracts should be sent are:

EXTENSION SECTION

Chairman: M. E. Senger, Department of Animal Industry, North Carolina State College, Raleigh.
Vice-Chairman: W. R. Van Sant, Department of Dairy Science, University of Arizona, Tucson.
Secretary: C. D. McGrew, Department of Dairy Science, Ohio State University, Columbus, 10.

PRODUCTION SECTION

Chairman: S. D. Musgrave, Department of Dairying, Oklahoma State University, Stillwater.
Vice-Chairman: J. C. Thompson, Ralston Purina Co., St. Louis, Missouri.
Secretary: L. H. Schultz, Department of Dairy Husbandry, University of Wisconsin, Madison.

MANUFACTURING SECTION

Chairman: Burdet Heinemann, Producers Creamery Co., Springfield, Missouri.
Vice-Chairman: M. L. Speck, Department of Animal Industry, North Carolina State College, Raleigh.
Secretary: D. M. Graham, Pet Milk Co., Research and Development Center, Greenville, Illinois.

(The Dairy Manufacturing Extension Section is a temporary subsection in the Manufacturing Section. R. B. Redfern, Dairy Extension, North Carolina State College, Raleigh, is chairman and W. S. Arbuckle, Department of Dairy Husbandry, University of Maryland, College Park, is secretary. All abstracts for papers in the Extension Section should be submitted through the regular channels of the Manufacturing Section, but should be identified for presentation at Extension Section meeting and a courtesy copy be sent to R. B. Redfern.)

BOOK REVIEW

BIOCHEMISTRY OF PLANTS AND ANIMALS. AN INTRODUCTION. M. Frank Mallette, Paul M. Althouse, and Carl O. Clagett. John Wiley and Sons, Inc., New York. 552 pp. 1960.

This book represents a publication of three members of the Department of Agricultural and Biological Chemistry of the Pennsylvania State University. Although the book is essentially new, it originated from a previous publication, Introduction to Agricultural Biochemistry, by Dutcher, Jensen, and Althouse, published in 1951, which, in turn, was derived from another of the same name published by Dutcher and Haley in 1932. The authors state that the book is intended to provide a general knowledge of biochemistry for students with basic training in inorganic and organic chemistry. Although it is intended primarily for students interested in the agricultural sciences, an effort has been made to orient the treatment broad enough for elementary courses intended for students from other disciplines. The subject matter is divided into three main sections, general biochemistry, plant biochemistry, and animal biochemistry, with appropriate subheadings, and concludes with an appendix containing some tables of nutrient requirements for human and farm animals and the chemical composition of common foods and feeds.

The scope of subjects covered is enormous and provides excellent reading material. In no case is a penetration made into a subject with any degree of fine detail. Specific references do not appear in the text but some suggested general references do appear at the end of each chapter. In most cases these references are to textbooks themselves. Other than in the historical section at the beginning, which traces the origin of agricultural science and physiological chemistry, chiefly up to about the turn of the century, no mention is made of eminent contemporary biochemists working in the field today. It is unfortunate that students who read this text will gain no appreciation of some of the people who have made and are making eminent contributions in the field of biochemistry and of some of the places where this work has taken place.

The authors have done an excellent job in distilling involved concepts down to a level that should be readily grasped by the student. Unfortunately, the lack of suitable background by the students for whom the book is intended has resulted in the necessity of making many statements of fact, rather than logically derived conclusions that will be really understood and retained. Even with this handicap, the authors have done a creditable job.

It is the reviewer's opinion that this book should serve as an excellent text in those Colleges of Agriculture that attempt to teach general biochemistry courses to large numbers of students with limited backgrounds.

B. L. LARSON
University of Illinois
Urbana, Illinois

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